Paediatric acute hepatitis of unknown aetiology: a national investigation and adenoviraemia case-control study in the UK

Sema Mandal, Ruth Simmons, Georgina Ireland, Andre Charlett, Monica Desai, Laura Coughlan, Annabel Powell, David Leeman, Christopher Williams, Claire Neill, Maureen C O'Leary, Clare Sawyer, Frances Rowley, Caroline Harris, Catherine Houlihan, Claire Gordon, Tommy Rampling, Helen Callaby, Katja Hoschler, Jade Cogdale, Erik Renz, Praveen Sebastianpilli, Catherine Thompson, Tiina Talts, Cristina Celma, Emma A Davies, Shazaad Ahmad, Nicholas Machin, Laura Gifford, Catherine Moore, Elizabeth M Dickson, Titus H Divala, David Henderson, Kathy Li, Philip Broadbent, Ines Ushiro-Lumb, Clare Humphreys, Tassos Grammatikopoulos, Jane Hartley, Chayarani Kelgeri, Sanjay Rajwal, Ifeanyichukwu Okike, Deirdre A Kelly, Malcolm Guiver, Ray Borrow, Renu Bindra, Alicia Demirjian, Kevin E Brown, Shamez N Ladhani, Mary E Ramsay, Declan T Bradley, Ardiana Gjini, Kirsty Roy, Meera Chand, Maria Zambon, Conall H Watson

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UK Health Security Agency, London, UK (S Mandal FFPH, R Simmons PhD, G Ireland PhD, A Charlett PhD, M Desai MDRes, L Coughlan MSc, A Powell BSc, D Leeman MFPH, C Houlihan FRCPath, C Gordon DPhil, T Rampling DPhil, H Callaby MRCP, K Hoschler PhD, J Cogdale MSc, E Renz MSc, P Sebastianpilli BSc, C Thompson PhD, T Talts BNatSci, C Celma PhD, I Ushiro-Lumb FRCPath, C Humphreys MFPH, R Borrow FRCPath, R Bindra FFPH, A Demirjian MD, K E Brown FRCPath, S N Ladhani MRCPH, M E Ramsay FFPH, M Chand FRCPath, M Zambon FRCPath, C H Watson FFPH); **UK Field Epidemiology Training Programme, UK Health Security Agency, London, UK** (D Leeman); **UK Field Epidemiology Training Programme, UK Health Security Agency, Cardiff, UK** (F Rowley MPH); **Public Health Wales, Cardiff, UK** (C Williams FFPH, C Sawyer MSc, F Rowley, C Harris MPH, L Gifford MSc, C Moore FRCPath, A Gjini FFPHM); **Public Health Agency, Belfast, UK** (C Neill MFPH, D T Bradley FFPH); **Public Health Scotland, Glasgow, UK** (M C O'Leary PhD, E M Dickson PhD, T H Divala PhD, D Henderson, P Broadbent MBChB,

Summary

Background An increase in acute severe hepatitis of unknown aetiology in previously healthy children in the UK in March, 2022, triggered global case-finding. We aimed to describe UK epidemiological investigations of cases and their possible causes.

Methods We actively surveilled unexplained paediatric acute hepatitis (transaminase >500 international units per litre) in children younger than 16 years presenting since Jan 1, 2022, through notifications from paediatricians, microbiologists, and paediatric liver units; we collected demographic, clinical, and exposure information. Then, we did a case-control study to investigate the association between adenoviraemia and other viruses and case-status using multivariable Firth penalised logistic regression. Cases aged 1–10 years and tested for adenovirus were included and compared with controls (ie, children admitted to hospital with an acute non-hepatitis illness who had residual blood samples collected between Jan 1 and May 28, 2022, and without known laboratory-confirmed diagnosis or previous adenovirus testing). Controls were frequency-matched on sex, age band, sample months, and nation or supra-region with randomised selection. We explored temporal associations between frequency of circulating viruses identified through routine laboratory pathogen surveillance and occurrence of cases by linear regression. SARS-CoV-2 seropositivity of cases was examined against residual serum from age-matched clinical comparison groups.

Findings Between Jan 1 and July 4, 2022, 274 cases were identified (median age 3 years [IQR 2–5]). 131 (48%) participants were male, 142 (52%) were female, and one (<1%) participant had sex data unknown. Jaundice (195 [83%] of 235) and gastrointestinal symptoms (202 [91%] of 222) were common. 15 (5%) children required liver transplantation and none died. Adenovirus was detected in 172 (68%) of 252 participants tested, regardless of sample type; 137 (63%) of 218 samples were positive for adenovirus in the blood. For cases that were successfully genotyped, 58 (81%) of 72 had Ad41F, and 57 were identified as positive via blood samples (six of these were among participants who had undergone a transplant). In the case-control analysis, adenoviraemia was associated with hepatitis case-status (adjusted OR 37·4 [95% CI 15·5–90·3]). Increases in the detection of adenovirus from faecal samples, but not other infectious agents, in routine laboratory pathogen surveillance correlated with hepatitis cases 4 weeks later, which independently suggested an association (β 0·06 [95% CI 0·02–0·11]). No association was identified for SARS-CoV-2 antibody seropositivity.

Interpretation We observed an association between adenovirus 41F viraemia and paediatric acute hepatitis. These results can inform diagnostic testing recommendations, clinical management, and exploratory in vitro or clinical studies of paediatric acute hepatitis of unknown aetiology. The role of potential co-factors, including other viruses and host susceptibility, requires further investigation.

Funding None

Introduction

In March, 2022, Scottish National Health Service (NHS) clinicians notified Public Health Scotland of five children aged 3–5 years with severe unexplained acute hepatitis, triggering a UK-wide investigation.[1] The UK paediatric tertiary liver units (Birmingham Women's and Children's NHS Foundation Trust, Leeds Teaching Hospitals NHS Foundation Trust, and King's College

Research in context

Evidence before this study

A rise in sudden-onset hepatitis in previously healthy children, resulting in many urgent paediatric transplant registrations, was observed in March, 2022, in the UK. With no evidence of viral hepatitis (hepatitis A–E) or travel history, the unexpected surge in cases resulted in an extensive investigation to determine the potential cause. A WHO alert in April, 2022, resulted in a further 35 countries reporting cases of acute hepatitis of unknown aetiology including transplants and deaths. The UK experience was one of the first international reports associated with this outbreak. Pathogen investigations identified adenovirus as a more frequently identified pathogen in case-patients, more commonly in blood and serum samples with adenovirus 41F. With similar findings in other countries, such as the USA, and parallel increases in adenovirus detection in routine surveillance, adenovirus was considered a possible cause. A literature search was undertaken in PubMed using the following terms "Hepatitis" AND "Adenoviridae Infections" AND "Humans" AND ("Child" OR "Child, Preschool") for the time period between Jan 1, 1964, and Dec 31, 2021, excluding papers focused on immunocompromised patients or hepatitis A. Only articles published in English were included. Few reports were found, suggesting fulminant hepatitis to be a rare complication of adenovirus infection in immunocompetent children.

Added value of this study

The findings from this case-control study, the first for this clinical syndrome, and active case finding and surveillance, provide evidence of an association between adenovirus 41F viraemia and paediatric acute hepatitis. Cases of acute hepatitis were 37 times more likely to be positive for adenovirus than the matched controls after adjusting for other factors. Time series regression analysis showed a temporal association between faecal adenovirus reports and acute hepatitis cases in young children, with an additional acute hepatitis case for about every 20 faecal adenovirus notifications. Additionally, our study findings did not support the role of SARS-CoV-2 infection as a major contributory co-factor to the clinical syndrome as SARS-CoV-2 was uncommon in our patients and we did not find higher rates of SARS-CoV-2 infection (by PCR or antibody positivity) in our patients than those observed in community infection surveys and age-matched comparison groups.

Implications of all the available evidence

These findings have led to a better understanding and description of severe non-A–E hepatitis in children, and informed clinical testing and management guidelines for paediatric acute hepatitis of unknown aetiology. Additionally, clinical surveillance of this syndrome has been established to ensure early detection of further cases. The findings have also focused efforts to improve adenovirus laboratory testing assays and algorithms, enhance the monitoring of seasonal trends in adenovirus in this population, and prompted investigation into adenovirus acquisition in this age group. Results to date have also stimulated further research to understand the contribution of adenovirus to severe acute hepatitis, specifically whether adenovirus acts as a trigger with co-factors such as other viruses (eg, adeno-associated virus 2 [AAV2]) and genetic susceptibility. Metagenomic investigations of UK cases, including a handful of individuals in the case-control study, have detected higher concentrations of AAV2 in liver, blood, plasma, and faecal samples, than observed in control groups. AAV2 requires a helper virus such as adenovirus to complete its lytic replication cycle and is known to be upregulated during liver damage. Further research is required to assess whether AAV2 reactivation or co-infection could lead to more severe hepatitis.

K Roy FFPH); **Manchester Medical Microbiology Partnership, Manchester Royal Infirmary, Manchester, UK** (E A Davies DClinSci, S Ahmad FRCPath, N Machin MSc, M Guiver PhD, R Borrow); **Belfast Health and Social Care Trust, Belfast, UK** (K Li FRCPath); **NHS Blood and Transplant, London, UK** (I Ushiro-Lumb); **King's College Hospital NHS Foundation Trust, London, UK** (T Grammatikopoulos MDRes); **Birmingham Women's and Children's NHS Foundation Trust, Birmingham, UK** (C Kelgeri MD, J Hartley MMedSci, D A Kelly CBE); **Leeds Teaching Hospitals NHS Trust, Leeds, UK** (S Rajwal MRCPCH); **Derbyshire Healthcare NHS Foundation Trust, Derby, UK** (I Okike PhD); **Evelina Children's Hospital, London, UK** (A Demirjian); **King's College London, London, UK** (A Demirjian); **University College London, UK** (C Houlihan); **Medical Sciences, University of Aberdeen, Aberdeen, UK** (H Callaby)

Correspondence to:
Dr Sema Mandal, UK Health Security Agency, London, NW9 5EQ, UK
Sema.Mandal@ukhsa.gov.uk

Hospital, NHS Foundation Trust) confirmed increases in weekly referrals and transfers during March and April, 2022. NHS Blood and Transplant reported as many super urgent paediatric liver transplant registrations in the first quarter of 2022 (January–March), as are usually reported annually. Notably, no corresponding increases in acute or fulminant hepatitis or liver transplants were reported in adults. Following a WHO alert on April 15, 2022, 35 countries reported 1010 childhood cases of acute hepatitis of unknown cause by July 8, 2022, including 46 transplants and 22 deaths.[2]

In children, mild, non-specific transient hepatitis with transaminitis is common in many viral infections, but severe or fulminant hepatitis is rare. A prospective study of paediatric infectious hepatitis hospital admissions in the UK and Ireland found that, in 2014, of 69 children who were admitted to hospital, 23% had viruses other than hepatitis A or B, and 33% remained unexplained; one of 39 children with these other viruses developed acute liver failure.[3] SARS-CoV-2 infection has also been associated with transient hepatitis in children and, rarely, acute liver failure.[4] Additionally, acute hepatitis might be a feature of multisystem inflammatory syndrome in children, which typically occurs 2–6 weeks after SARS-CoV-2 infection.[5]

The first US cases identified were a cluster of nine cases associated with adenoviraemia,[6,7] prompting active surveillance nationally, with 296 cases identified between October, 2021, and June, 2022, of which 18 required liver transplants and 11 died.[8] Detection of adenovirus early in UK investigations, including viraemia with adenovirus 41F, and concurrent increases in adenovirus detection in routine surveillance of laboratory notifications, established this as a possible cause.

For the 2022 outbreak of acute hepatitis of unknown aetiology in the UK, we aimed to epidemiologically investigate and characterise the acute hepatitis cases through active case-finding; to investigate adenovirus as

a possible cause of acute hepatitis through a national case-control study; to evaluate the temporal association between the occurrence of acute hepatitis cases and frequency of enteric and respiratory viruses identified through routine laboratory surveillance; and to evaluate the association between acute hepatitis cases and SARS-CoV-2 antibody positivity.

Methods

Study design and participants

In this epidemiological surveillance and case-control study, active case-finding commenced in early April, 2022, when UK public health agencies cascaded an alert asking paediatricians, microbiologists, and paediatric liver units to notify and complete a reporting form for children fulfilling the following outbreak case definition: a person younger than 16 years presenting since Jan 1, 2022, with an acute hepatitis not due to hepatitis A–E viruses, or an expected presentation of metabolic, inherited or genetic, congenital, or mechanical cause with serum transaminase (aspartate aminotransferase [AST] or alanine aminotransferase [ALT]) more than 500 international units per litre (IU/L). Children with incomplete hepatitis serology or transaminase results were classified as pending and, for cases in which it was possible, followed-up to resolve.

For the case-control study, only cases resident in the UK and fulfilling the case definition were included. Cases included in the case-control study were restricted to those age 1–10 years to reduce potential mis-classifications from infant and adolescent causes of hepatitis. Children not tested for adenovirus were excluded. Data were collected on controls presenting between Jan 1 and May 28, 2022. Control blood samples were obtained from and tested at national reference laboratories. These were residua from otherwise healthy children admitted to hospital with an acute non-hepatitis illness, specifically those whose clinical investigations included testing for invasive meningococcal disease. Testing for invasive meningococcal disease is part of the routine investigation of a serious acute febrile illness in a young child, for which most cases that undergo testing do not have meningococcal disease,[9] and testing is undertaken for diagnostic exclusion purposes. Alongside their clinical appropriateness as controls, samples for meningococcal testing routinely use whole blood, which is the preferred analyte for this hepatic syndrome. Higher adenovirus detection sensitivity in whole blood compared with plasma or serum has been noted in both UK and US investigations,[8,10] supporting these residual samples taken from acutely unwell children being an appropriate control sample set. Controls were frequency-matched to cases on sex (at birth), age band (age 5 years or younger, and older than 5 years), sample months, and nation or supra-region with randomised selection. Controls with known laboratory-confirmed diagnosis (eg, *Neisseria meningitidis*) or previous adenovirus testing were excluded. The protocol of this case-control study has previously been published.[11] Data on sex or gender was obtained from laboratory or medical records or case notification forms, which were entered by the clinical teams. Sex is assumed to be sex at birth (unless stated otherwise), which is reasonable as the majority of cases and controls are all young children.

See Online for appendix

Routine laboratory pathology surveillance data across all four UK nations, from 2019 onwards, were reviewed for trends and exceedances, including pathogens associated with acute hepatitis. For analysis of temporal associations between acute hepatitis and circulating viruses (adenovirus, enterovirus, SARS-CoV-2, human metapneumovirus [hMPV], rhinovirus, and rotavirus), laboratory data from children aged 10 years and younger in England from late 2021 through early 2022 were used. In Wales, laboratory reports of respiratory adenovirus in those younger than 25 years were used to compare adenovirus positivity between between 2022 and earlier years.

To investigate previous SARS-CoV-2 infection by comparing antibody positivity, serum from children with acute hepatitis (aged 1–10 years), whose samples were available at UKHSA reference laboratories, were included. Age-matched comparison group samples from NHS patients, collected from January to May 2022, were obtained from UKHSA's Seroepidemiology unit, which holds a collection of anonymous, unlinked residual sera from NHS virology laboratories.

UK public health agencies have permission to process patient confidential information for national surveillance of communicable diseases under: Regulation 3 of the Health Service Regulation 2002 for England, the Public Health (Scotland) Act 2008 and the NHS Scotland Act 1978 for Scotland, and the Public Health Wales National Health Service Trust (Establishment) Order 2009 for Wales. The case-control study was deemed to be part of the outbreak response and no further NHS permissions were required by the UK Health Security Agency (UKHSA) Research Ethics Governance Group for England, Scotland, and Northern Ireland, and the Public Health Wales Research and Development Office for Wales (appendix p 15). The study was reviewed by the UKHSA Research Ethics and Governance Group as part of the Research Support and Governance Office who confirmed the work was neither treatment nor research, and covered by Regulation 3 of The Health Service (Control of Patient Information) Regulations 2002, therefore would not require informed consent. This review was also undertaken in each UK country.

Procedures and outcomes

Most cases were initially tested or had confirmatory testing for adenovirus at a single UKHSA laboratory using the adenovirus testing method based on Heim and colleagues,[12] targeting the Hexon gene and followed by partial or whole genome sequencing to establish genotype.

In Northern Ireland, adenovirus was tested using a quantitative commercial assay (RealStar, Adenovirus PCR Kit). In Scotland. Adenovirus testing was a multiplex real-time PCR based on van Maarseveen and colleagues.[13] In Wales, Seegene multiplex qualitative PCR was used for adenovirus, human herpesvirus 6, and human herpesvirus 7. Test choice and platforms vary widely outside of the UKHSA, and not all cases, particularly those identified early in the incident, will have been tested for all recommended pathogens (appendix p 6).

For all cases, parents were interviewed with a trawling questionnaire to identify symptoms, common exposures (pets, toxins, food, drugs), and preceding illnesses in the family and close contacts. As the investigation evolved, a focused, enhanced questionnaire asked about specific foods, nursery or childcare attendance before and during the COVID-19 pandemic lockdowns, hospital admissions, and outcome at 28 days.

Additionally, in England, all reported cases were electronically linked to the UKHSA's national Unified Sample Dataset (which captures SARS-CoV-2 results), the Second Generation Surveillance System (for pathogen diagnoses), National Immunisation Management Service (for SARS-CoV-2 vaccinations), Hospital Episode Statistics (for missing hospital admission data), and the UKHSA reference laboratory (for samples submitted for diagnostic or confirmatory testing for viral hepatitis, including hepatitis E serology).

SARS-CoV-2 PCR test at presentation was defined by a respiratory sample at the earliest presentation date (identified through notifications, questionnaires, or Hospital Episode Statistics) within 2 days either side of the earliest presentation date. SARS-CoV-2 tests less than 14 days or between 14–90 days of earliest date of acute hepatitis presentation, were identified in the Unified Sample Dataset and the Second Generation Surveillance System. Identification of other pathogens within 2 days of earliest presentation date was through the Second Generation Surveillance System, notifications, or samples submitted to reference laboratories. SARS-CoV-2 serology results were obtained through notifications, the Second Generation Surveillance System, and testing at UKHSA laboratories. Nucleocapsid (N) or Spike (S) antibody positivity was considered indicative of past infection because cases were highly unlikely to be vaccinated against SARS-CoV-2, consistent with age-based recommendations.

For the case-control study, control samples were tested for adenovirus and, for cases in which it was possible, human herpesvirus 6 and human herpesvirus 7. SARS-CoV-2 PCR test results for controls in England were obtained from the Unified Sample Dataset and the Second Generation Surveillance System.

Regarding laboratory surveillance, profiles of tests performed routinely at different hospitals vary and arrangements for data capture of positive and negative test results also vary in different UK territories. Data on both negative and positive results are available for all Welsh NHS laboratories; however, only positive results are reported in England, Scotland, and Northern Ireland. Laboratories report confirmation of both notifiable and non-notifiable organisms to their relevant public health agency; voluntary reporting arrangements for non-notifiable organisms, such as adenovirus, vary. Adenovirus testing can be discretionary as part of multiplex PCR testing of respiratory or faecal specimens. Children admitted to hospital in Wales with respiratory symptoms usually have a full respiratory screen that includes adenovirus. Within these different arrangements, routine laboratory data across all four UK nations were reviewed for trends and exceedances, including pathogens associated with acute hepatitis, identified in any sample type, and for adenovirus, also in faecal and respiratory samples separately.

To investigate previous SARS-CoV-2 infection, serum submitted to UKHSA laboratories from some cases with acute hepatitis, and unlinked, anonymous, age-matched control samples from the UKHSA Seroepidemiology Unit were tested at UKHSA laboratories for SARS-Co-V-2 N and S antibodies (Roche diagnostics, IN, USA).

Statistical analysis

All summary statistics for data were presented as follows: categorical data were presented as absolute values with percentages and continuous data (eg, age) were presented as medians, IQR, and range. In regression analyses, odds ratios (ORs) were presented with 95% CIs. Among the cases, characteristics between adenovirus-positive and adenovirus-negative cases were compared by χ^2 or Fisher's exact test for proportions and the Mann-Whitney test for medians. Proportions were presented where data were reported (ie, with missing data excluded).

The case-control study was designed to test the primary hypothesis that adenovirus infection, particularly viraemia, was associated with non A–E hepatitis in the current outbreak. Secondarily we explored associations with current (during hospitalisation or up to 14 days prior) or past (14 to 90 days previously) PCR-confirmed SARS-CoV-2 infection, or human herpesvirus 6 and human herpesvirus 7 infection. Characteristics of included cases were compared with the main case cohort and with controls using χ^2 or Fisher's exact tests for proportions and the Mann-Whitney test for medians. Primary analysis was on adenovirus testing on whole blood samples only.

Sensitivity analysis included cases with adenovirus tested in any sample type (whole blood, plasma, and serum) and serum controls. We also did a sensitivity analysis restricted to data from England, for which ethnicity data were available, but were not available for other UK countries.

Due to small case numbers, the study size was based on the minimum detectable odds ratios (ORs) for

80% power, 5% significance level, and percentage of controls being exposed between 5–15%. Assuming a control-to-case ratio of 3:1, 33–69 cases would be needed to detect an OR of 4 or more. To address potential insufficient or missing blood samples in selected controls, 4:1 sampling was used. Unconditional multivariable Firth penalised logistic regression was done with demographic variables used in the frequency matching included as predictor variables to account for any residual confounding. The Firth penalised logistic regression model adjusted for sex, age, month of presentation, region, and adenovirus DNA result, all categorical variables. The biases from ignoring the frequency-matched design in the analysis are relatively small and similar in magnitude to those seen on conditional analyses.[14] Firth penalisation handles both sparse data biases and possible separation affecting OR estimation in case-control studies with small numbers.[15] Interactions were investigated between adenovirus and age, human herpesvirus 6, human herpesvirus 7, and SARS-CoV-2, which were not found to be significant. Effect modification with SARS-CoV-2, human herpesvirus 6, and human herpesvirus 7 was explored by including interactions into models, noting the study was not powered to detect effect modification. A conditional logistic regression sensitivity analysis was performed using the frequency-matched sets.

In England, for children aged 10 years or younger, temporal correlations between weekly acute hepatitis case counts and circulating viruses were investigated. Circulating viruses included were adenovirus, SARS-CoV-2, enterovirus, rhinovirus, hMPV, and respiratory syncytial virus in any sample type (mostly faecal or respiratory), and for adenovirus by any sample type, and presented by faecal and respiratory samples separately. Cross-correlations with a range within 4-week leads or lags were plotted, and time-series analysis were done using linear regression models fitted using the week with the strongest correlation.

In Wales, age-stratified (<5, 5–9, 10–24 years) adenovirus positivity in patients with a full respiratory viral screen was compared for the January–May period between 2017–19 and 2022 by a two-proportion Z-test.

A χ^2 test was used to compare SARS-CoV-2 N and S antibody proportions between acute hepatitis cases and residual control samples collected by the UKHSA seroepidemiology unit. Data were analysed with STATA software (version 15).

Role of the funding source

There was no funding source for this study.

Results

Between Jan 1 and July 4, 2022, 274 children younger than age 16 years fulfilled the case definition, with 261 (95%) aged 10 years or younger (table 1); 195 (71%) resided in England, 36 (13%) in Scotland, 19 (7%) in Wales, and 24 (9%) in Northern Ireland. 131 (48%) participants were male, 142 (52%) were female, and one (<1%) had sex data unknown. Among cases in England and Scotland the median age was 3 years (IQR 2–5). For cases in which ethnicity was reported, 224 (91%) of 246 were White. Jaundice (195 [83%] of 235) and gastrointestinal symptoms (diarrhoea, vomiting, or abdominal pain; 202 [91%] of 222) were the most common presentations. Overall, 246 (97%) of 253 were admitted to hospital, 15 (5%) required liver transplantation, and none died. Four (1%) children had received COVID-19 vaccination. No clear food, environmental, or toxin exposures were identified.[16] Among cases born in 2017–19 (aged 2–5 years), of the 67 with data, 16 (24%) attended nursery before March, 2020, and 12 (18%) attended nursery between March, 2020, and February, 2021, including five new attenders.

Adenovirus was the most common pathogen identified at admission, regardless of sample type, identified in 172 (68%) of 252 participants (table 1). Adenovirus was identified in 137 (63%) of 218 blood samples, in 33 (27%) of 124 faecal samples, and in 26 (23%) of 115 respiratory samples. 30 (31%) of 98 blood samples were positive for human herpesvirus 6 and 33 (38%) of 88 blood samples were positive for human herpesvirus 7, while 22 (9%) of 237 throat or respiratory samples were SARS-CoV-2 PCR-positive. SARS-CoV-2 antibodies were presented in 65 (61%) of 106 cases. 13 (87%) of 15 children who received a liver transplant were adenovirus-positive by any sample type. Among the cases, a higher proportion of adenovirus-positive children than adenovirus-negative children were aged 5 years or younger and required transplantation (appendix p 7). Median peak transaminase (ALT/AST) and bilirubin concentrations were higher in adenovirus-positive than adenovirus-negative cases ($p<0·0001$; appendix p 7). There was no difference in proportions with abnormal clotting (international normalised ratio >2 or prothrombin time >14 seconds) between the two groups (appendix p 7).

Adenovirus peak viral load in blood samples was reported for 73 cases in England (median 9590 copies per mL [IQR 3240–27 407; range 10–318 200]) and was 7·0 times higher in those who underwent liver transplant than those who did not (56 426 copies per mL *vs* 8050 copies per mL; $p=0·0090$).

Among adenovirus-positive samples from participants who resided in England that were successfully subtyped, 58 (81%) of 72 were 41F (51 from whole blood, one from serum, five from unspecified blood, and one from an unknown sample type). Other subtypes included adenovirus types 1, 2, and 5. Adenovirus 41F was not detected in faecal samples, even when detected in blood; occasionally other adenoviral subtypes were detected in faecal or respiratory material (table 1). In transplanted cases, subtyping (whole blood) found six cases of subtype 41F with one typing failure.

	All cases identified through active case finding (n=274)	Cases included in case-control study primary analysis (n=73)
Sex		
Male	131/273 (48%)	34 (47%)
Female	142/273 (52%)	39 (53%)
Unknown	1	0
Age group, years		
≤5	214/273 (78%)	66/73 (90%)
6–10	47/273 (17%)	7/73 (10%)
11–15	12/273 (4%)	..
Median (IQR; range)*	3 (2–5; 0–15)	3 (3–4; 1–9)
Month of presentation		
January–February	43/266 (16%)	18/73 (25%)
March–April	154/266 (58%)	49/73 (67%)
May–June	69/266 (26%)	6/73 (8%)
Country or region		
England	195/274 (71%)	57 (78%)
East Midlands	18/190 (9%)	3/57 (5%)
East of England	21/190 (11%)	5/57 (9%)
London	18/190 (9%)	2/57 (4%)
North East	17/190 (9%)	5/57 (9%)
North West	26/190 (14%)	7/57 (12%)
South East	29/190 (15%)	9/57 (16%)
South West	20/190 (11%)	10/57 (18%)
West Midlands	21/190 (11%)	9/57 (16%)
Yorkshire and the Humber	20/190 (11%)	7/57 (12%)
Wales	19 (7%)	13 (18%)
Northern Ireland	24 (9%)	NA
Scotland	36 (13%)	3 (4%)
Ethnicity		
White	224/246 (91%)	68/72 (94%)
Non-White	22/246 (9%)	4/72 (6%)
Unknown	28	1
Reported symptoms		
Jaundice	195/235 (83%)	50/55† (91%)
Pale stool or dark urine	115/136 (85%)	42/48† (88%)
Gastrointestinal‡	202/222 (91%)	52/54† (96%)
Fever	60/182 (33%)	15/49† (31%)
Respiratory	53/195 (27%)	16/50† (32%)
Other§	172/214 (80%)	43/50† (86%)
Highest level of care		
Not admitted to hospital	7/253 (3%)	1/57† (2%)
Admitted to hospital	246/253 (97%)	56/57† (60%)
Paediatric intensive care unit	68/246 (28%)	16/56† (29%)
Received a transplant	15/274 (5%)	6/57† (11%)
Nursery or school attendance in cases born 2017–19†		
Before the lockdown in March, 2020	16/67 (24%)	11/36 (31%)†
Pathogens at time of presentation or admission		
AdV DNA (any sample) detected	172/252 (68%)	64/73 (88%)
Detected in blood or serum	137/218 (63%)	..
Detected in faeces	33/124 (27%)	..
Detected in respiratory samples	26/115 (23%)	..
SARS-CoV-2 RNA	22/237 (9%)	5/59 (8%)
Human herpesvirus 6 DNA	30/98 (31%)	16/34 (47%)
Human herpesvirus 7 DNA	33/88 (38%)	8/19 (42%)
SARS-CoV-2 antibodies¶	65/106 (61%)	..
AdV subtype 41F†	58/72 (81%)‖	28/29 (97%)
Detected in whole blood†	57/57‖ (100%)	..
Other adenovirus subtypes detected†	19/72** (26%)	..
COVID-19 vaccination		
One or more doses	4/274 (1%)	..

(Table 1 continues in next column)

(Continued from previous column)

Data are n (%) or n/N (%), unless otherwise specified. AdV=adenovirus. NA=not available. Ethnicity was reported by paediatricians or parents (based on their responses to the questionnaire), or through linking to hospital records. *England and Scotland only. †England only. ‡Vomiting, diarrhoea, or abdominal pain. §Nausea, loss of appetite, conjunctivitis, rash, bloody stool, lethargy, or malaise. ¶England and Northern Ireland. ‖Includes cases with co-infection of Ad41F in blood and another subtype in a different sample: two cases with Ad2C in faeces, one case with Ad2C in faeces and Ad2C in throat swab, and one case with Ad1C in faeces. **Other subtypes included adenovirus types 1, 2, and 5.

Table 1: **Characteristics of UK cases from active case-finding and the case-control study, January–June, 2022**

For the case-control study, of the 110 cases aged 1–10 years and 326 controls, 37 cases and 82 controls (including Northern Ireland samples) were excluded from the primary analysis as they did not have whole blood samples; a further 72 controls were excluded due to insufficient samples. Thus, 73 cases, representing 27% of the incident cohort, and 172 controls (ratio 1:2·4) were analysed. Included controls had similar demographic characteristics to those with insufficient samples. Cases had a higher proportion of participants who were younger than age 5 years, presentation in January and February, transplantations, and adenovirus and human herpesvirus 6 or human herpesvirus 7 positivity than did the active case-finding cohort, but were similar for other characteristics (table 1).

There were no important differences in demographics between cases and controls (table 2). Adenovirus positivity was higher in cases than controls (88% [64 of 73] *vs* 15% [25 of 172]; p<0·0001; table 2). SARS-CoV-2 positivity in cases at admission was similar to positivity in controls (8% (five of 59) *vs* 12% [eight of 69], p=0·56), and among cases in participants who resided in England

	Cases (n=73)	Controls (n=172)	p value
Sex			
Male	34 (47%)	94 (55%)	0·25
Female	39 (53%)	78 (45%)	..
Age group, years			
1–5	66 (90%)	149 (87%)	0·41
6–10	7 (10%)	23 (13%)	..
Median age (IQR; range)	3 (3–4; 1–9)	3 (2–5; 1–10)	..
Month of presentation			
January–February	18 (25%)	54 (31%)	0·001
March–April	49 (67%)	117 (68%)	..
May–June	6 (8%)	0	..
Region			
North England	19 (26%)	52 (30%)	0·41
Midlands (England)	12 (16%)	22 (13%)	..
South England	26 (36%)	45 (26%)	..
Wales	13 (18%)	45 (26%)	..
Northern Ireland*	..	..	..
Scotland	3 (4%)	8 (5%)	..
Deprivation index decile (with 1 being the most deprived)			
Median (IQR; range)	4 (2–7; 1–10)	4 (2–7; 1–10)	..
Ethnicity			
White	68/72 (94%)	96/116 (83%)	0·24
Non-White	4/72 (6%)	20/116 (17%)	..
SARS-CoV-2			
Vaccination	1 (1%)	1 (<1%)	0·53
Adenovirus DNA whole blood			
Detected	64 (88%)	25 (15%)	<0·0001
Not detected	9 (12%)	147 (85%)	..
SARS-CoV-2 RNA at admission			
Detected	5/59 (8%)	8/69 (12%)	0·56
Not detected	54/59 (92%)	61/69 (88%)	..
SARS-CoV-2 RNA within 14 days of admission†			
Detected	5/54 (9%)	8/79 (10%)	0·87
Not detected	49/54 (91%)	71/79 (90%)	..
SARS-CoV-2 RNA between 14 and 90 days of admission†			
Detected	3/12 (25%)	18/35 (51%)	0·11
Not detected	9/12 (75%)	17/35 (49%)	..
Human herpesvirus 6 DNA			
Detected	16/34 (47%)	63/144 (44%)	0·73
Not detected	18/34 (53%)	81/144 (56%)	..
Human herpesvirus 7 DNA			
Detected	8/19 (42%)	49/119 (41%)	0·94
Not detected	11/19 (58%)	70/199 (59%)	..
SARS-CoV-2 antibodies†			
Detected	21/33 (64%)	..	..
Not detected	12/33 (36%)	..	..

Data are n (%) or n/N (%), unless otherwise indicated. Only whole blood samples were included in the primary analysis. Ethnicity was reported by paediatricians or parents or through linking to hospital records. P values represent whether there is a significant difference between cases and controls by the dichotomous or categorical variables. *Northern Ireland samples were not whole blood and therefore were not included in the primary analysis of the case-control study. †England only.

Table 2: **Demographic characteristics of UK cases and controls in case-control study, January–June, 2022**

in the 14–90 days before hospital admission (25% [three of 12] *vs* 51% [18 of 35]; p=0·11; table 2). Although testing was incomplete, there was no difference in human herpesvirus 6 (47% [16 of 34] *vs* 44% [63 of 144]; p=0·73) and human herpesvirus 7 (42% [eight of 19] *vs* 41% [49 of 119]; p=0·94) positivity between cases and controls (table 2). SARS-CoV-2 antibody positivity was 64% (21 of 33) for cases but it was not possible to test control samples for SARS-CoV-2 antibodies.

In multivariable analyses, adjusting for all other factors, adenovirus positivity in the blood remained an independent factor associated with acute hepatitis (adjusted OR 37·4 [95% CI 15·5–90·3]; table 3). Although human herpesvirus 6 and human herpesvirus 7 were not associated with acute hepatitis in the univariable analysis, their inclusion increased the strength of association between acute hepatitis cases and adenovirus, but not sufficiently enough to suggest confounding. Stratifying by human herpesvirus 6, there was some evidence of effect modification with the estimated OR for adenovirus in those testing positive (adjusted OR 47·4 [95% CI 6·1–367·9]) and negative (adjusted OR 15·5 [95% CI 4·0–60·5]) for human herpesvirus 6 (test of interaction p=0·53), indicating this might be due to chance and therefore human herpesvirus 6 was not included in the multivariable model (appendix pp 9–10). There was no evidence of effect modification for human herpesvirus 7 (adjusted OR 71·0 [95% CI 2·7–1843·28] *vs* adjusted OR 28·7 [3·1–261·6]; test of interaction p=0·61). There was no interaction between adenovirus and age at diagnosis (p=0·42); however, there was some evidence of increasing risk of acute hepatitis with increasing age, limited by sparse data in older age groups (appendix p 11).

In the sensitivity analysis restricted to England data, which included ethnicity data and whole blood samples for cases (n=57) and controls (n=119), adenovirus remained a significant independent factor (adjusted OR 36·7 [95% CI 13·4–100·8); ethnicity was not significant (appendix p 12).

A sensitivity analysis using conditional (fixed-effects) logistic regression found a similar increased odds of adenovirus detection among cases compared with controls (OR 43·0 [95% CI 16·0–115·4]; appendix p 13). Results were similar when expanding analysis to include non-whole blood samples (plasma and serum; OR 25·9 [95% CI 12·8–52·5]), albeit with lower odds than the primary analysis restricted to whole blood (appendix p 14).

In laboratory surveillance, an increase in adenovirus detections in respiratory and faecal samples was observed in the UK from December, 2021 (figure; appendix p 3). There was a positive correlation between adenovirus faecal sample surveillance detections and hepatitis cases with a 4-week lag (correlation coefficient 0·48; figure) but not for COVID-19, hMPV, respiratory syncytial virus, rhinovirus, rotavirus, or enterovirus, for which increases in those

age 10 years or younger have been noted since December, 2021 (appendix pp 3–5). Linear regression time series analysis showed that adenovirus detections in faecal samples 4-weeks previously significantly predicted acute hepatitis cases (β 0·06 [95% CI 0·02–0·11]; R^2=0·30; p=0·01; for about every 20 faecal adenovirus notifications there was one additional acute hepatitis case (appendix pp 4–5). In Wales, in addition to increases in testing and adenovirus detection in children, respiratory adenovirus positivity in those younger than 5 year was higher in 2022 than in 2017–19 (18·9% *vs* 11·7%; p<0·0001), but not in older age groups (appendix p 8).

For the SARS-CoV-2 seropositivity comparison between cases and age-matched, contemporaneous, controls, serum seropositivity was 60% (26 of 43) from those aged 1–4 years and 67% (12 of 18) from those aged 5–10 years, compared with 47% (101 of 215) and 70% (289 of 412), in age-matched controls (not statistically significant; appendix p 2).

Discussion

In children, the cause of acute hepatitis often remains unknown. The surge in cases with fulminant liver failure in early April, 2022, was unexpected and concerning. In this UK evaluation, having excluded the viral hepatitis types A–E, additional testing found adenovirus in 68% of cases, with infrequent identification of human herpesvirus 6, human herpesvirus 7, and SARS-CoV-2. Analysis of laboratory surveillance data, case reports and laboratory investigations, and a case-control study identified a strong association between adenovirus and acute hepatitis, but limited evidence for association with other infections. Adenovirus in cases was mainly detected in blood, and typing in cases frequently identified subtype 41F, an enteric adenovirus associated with gastrointestinal illness.[17] The high prevalence of adenoviraemia in hepatitis cases was not found in age-matched acutely unwell hospital controls.

Scrutiny of laboratory surveillance reports found that trends in faecal adenovirus detection in children coincided with subsequent trends in hepatitis cases. Faecal adenovirus detections were higher than pre-pandemic levels and were not likely to be due to increased testing as they preceded hepatitis alerting. Time-series analysis in England showed a positive correlation between previous faecal adenovirus laboratory reports and acute hepatitis cases, which was not seen for other pathogens. In Wales, increased respiratory adenovirus positivity was also observed to correspond temporally with the surge in acute hepatitis cases of unknown origin, consistent with previous demonstration that gastrointestinal adenoviral infections are detectable in respiratory samples.[18]

Compared with other countries, the UK reported the highest incidence of acute hepatitis cases, with possible higher case ascertainment through centralised surveillance and national clinical referral pathways for specialist care.[2,8,19] In the US hepatitis cases, adenoviraemia detection was also higher than was SARS-CoV-2 detection, with no other common exposures identified, and Ad41F was identified as the most frequent subtype.[6–8,20] The US cases had a similar clinical profile to our UK cases—a subset of which was published recently—with jaundice, diarrhoea, and vomiting showing as common presentations.[21] Additionally, our transplanted cases had more abnormal liver and clotting profiles, and higher adenovirus viral loads than did non-transplanted cases.[21] Although the UK

	Univariable	Multivariable
Sex		
Male	1 (ref)	1 (ref)
Female	1·4 (0·8–2·4)	1·1 (0·5–2·5)
Age, years		
1	1 (ref)	1 (ref)
2	2·3 (0·7–7·8)	1·5 (0·3–7·1)
3	4·4 (1·5–13·5)	7·8 (1·8–33·3)
4	4·0 (1·3–12·6)	7·6 (1·0–20·7)
5–10	2·3 (0·7–7·3)	4·0 (0·9–17·7)
Month of presentation		
January–February	1 (ref)	1 (ref)
March–April	1·2 (0·7–2·3)	0·8 (0·3–1·9)
May–June	38·3 (2·1–713·2)	6·6 (0·3–157·4)
Region		
North England	1 (ref)	1 (ref)
Midlands (England)	1·5 (0·6–3·6)	1·1 (0·3–3·8)
South England	1·6 (0·8–3·2)	1·6 (0·6–4·3)
Wales	0·8 (0·4–1·8)	0·5 (0·1–1·9)
Northern Ireland*	..	..
Scotland	1·1 (0·3–4·3)	1·7 (0·02–178·8)
Deprivation		
Decreasing deprivation decile from 1 (most deprived) to 10 (least deprived)	1·0 (0·9–1·1)	1·0 (0·9–1·2)†
Adenovirus DNA		
Detected	39·3 (17·6–87·4)	37·4 (15·5–90·3)
Not detected	1 (ref)	1 (ref)
Human herpesvirus 6 DNA		
Detected	1·3 (0·6–2·7)	1·2 (0·4–3·3)†
Not detected	1 (ref)	1 (ref)
Human herpesvirus 7 DNA		
Detected	1·1 (0·4–2·7)	1·4 (0·4–5·1)†
Not detected	1 (ref)	1 (ref)
SARS-CoV-2 RNA at presentation		
Detected	0·7 (0·2–2·3)	1·8 (0·4–9·0)†
Not detected	1 (ref)	1 (ref)

Data are OR (95% CI). The multivariable model was adjusted for sex, age, month of presentation, region, and adenovirus DNA result (all categorical variables). OR=odds ratio. *Northern Ireland samples were not whole blood and therefore were not included in the primary analysis of the case-control study. †Variables not included in the final model as they were no longer significant when adjusting for other variables.

Table 3: **Univariable and multivariable unconditional logistic regression for case-control analysis in the UK, January–June, 2022**

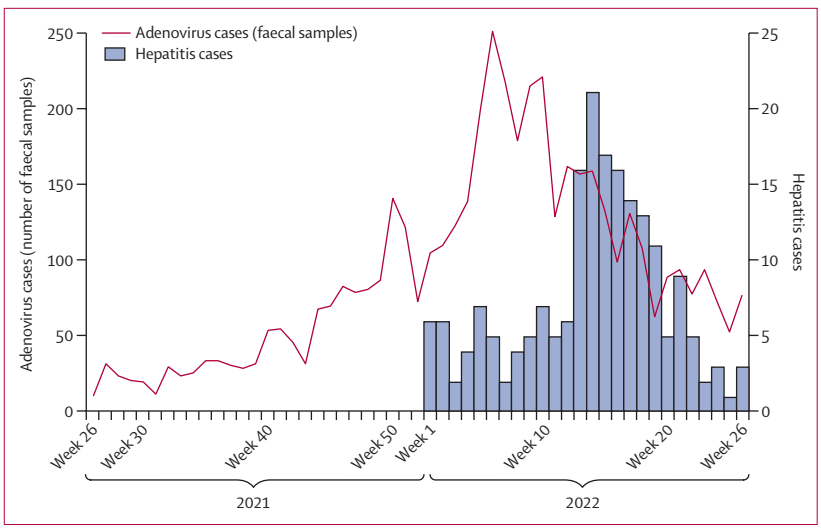

Figure: **Adenovirus detections from faecal samples in laboratory surveillance and cases of acute hepatitis with unknown aetiology, England, from week 26, 2021, to week 26, 2022**

and US case-patients were predominantly healthy children, adenoviraemia associated with acute hepatitis has been described mainly in immunocompromised children,[22] but this might be because adenovirus testing is not routinely done for hepatitis. Further investigation is warranted, including on the pathogenesis of adenovirus-associated hepatitis, the possible contribution of other co-factors, and the potential role of human leukocyte antigen (HLA) mediated susceptibility to a severe hepatitis presentation.[23]

Our investigations provide evidence that adenovirus 41F infection is likely to be implicated in the cause of acute hepatitis, corresponding to Bradford Hill's criteria for causality[24] for effect size, consistency,[6] specificity, and temporality. However, it remains unclear why this surge in cases happened recently and why some children developed such fulminant liver failure. A novel strain of Ad41F is unlikely as sequencing of Ad41F detected in cases indicates similarity with background strains in circulation.[11] An alternative hypothesis is that the UK experienced an exceptionally large wave of adenovirus infections, as evidenced by the surveillance data, causing a very rare or under-recognised complication to manifest. It is plausible that the pandemic lockdowns reduced mixing in nursery-type settings delaying first exposure to adenovirus and potentially other co-circulating hepatotropic viruses. Our cohort might also have had lower nursery attendance pre-pandemic (potentially delaying exposure further): 24% of relevant-age cases compared with 64% in a 2019 England survey.[25] Even fewer relevant-age cases attended or started nursery during the pandemic (no 2020 national comparator available). Enteric adenovirus most commonly affects those younger than 2 years and is rare in those older than 4 years.[26] Later exposure could lead to dysregulated immune responses and more severe presentations of a common infection in susceptible children, as is observed with other viruses (eg, varicella, measles, and hepatitis A). This hypothesis is in keeping with the distribution of ages in our hepatitis case-cohort and the association with increasing age observed in our case-control study.

Adeno-associated virus 2 (AAV2), which is usually acquired in early childhood, has been detected via metagenomics in blood and explanted livers of some cases, and AAV2 reactivation or co-infection with adenovirus might lead to more severe hepatitis.[23,27] AAV2 replication requires helper viruses (eg, adenovirus, herpes simplex virus) and is known to be upregulated during liver damage;[28,29] however, assessing causality requires further research. Human herpesvirus 6 and human herpesvirus 7 primary infection occurs in the first 2 years of life,[30] and positivity in hepatitis cases might reflect persistent infection or nucleic acid detection rather than acute infection, although our findings do not exclude a role in some case-patients, consistent with background rates. Further genomic investigations of cases within the UK, including individuals in the case-control study, identified high concentrations of AAV2 in liver, blood, plasma, and stool samples and very low concentrations of adenovirus DNA, whereas little or no AAV2 were found in comparison groups.[23,27] Similar findings have also been reported in the USA.[31] Ho and colleagues[23] discuss an association between AAV2 and the II HLA allele DRB1*04:01, for which the varied distribution of the HLA allele between ethnicities and geographical areas could inform the differences in where incidents were reported, and the higher proportion of cases of White ethnicity compared with the underlying distribution among children in the UK of the same age.[23]

SARS-CoV-2 infection can be associated with elevated liver enzymes and rarely, acute liver failure,[32,33] but acute SARS-CoV-2 infection was uncommon in our cohort and other acute hepatitis cohorts.[8] Additionally, SARS-CoV-2 infection rates in our cases were similar to community infection rates in similar age groups, and SARS-CoV-2 PCR positivity in our cases was similar to an age-matched random control sample of children presenting to emergency departments between January and June, 2022.[34] Alternatively, a post-infectious, immune-mediated inflammatory syndrome affecting the liver, akin to multisystem inflammatory syndrome in children,[4] has also been hypothesised because of high SARS-CoV-2 Omicron case numbers in young children in the UK in early 2022. However, SARS-CoV-2 antibody positivity—a marker of prior infection—in our cases was similar to seroprevalence in an age-matched comparison group.

Our findings are limited by the use of mainly observational real-world data. Differential propensity of clinicians to report cases might have affected case ascertainment, and some cases might have been missed if they did not fulfil inclusion criteria. Variation in testing assays, algorithms, and sample type might contribute to differences in adenovirus detection between UK nations.

Time-series analysis had an R^2 of 0·30, which suggests considerable unexplained variability remains. Inconsistent testing for adenovirus and the lower sensitivity of non-whole blood specimens[10] could have underestimated adenovirus positivity among our cases. SARS-CoV-2 positivity included PCR confirmation through electronic record linkage so bias could be introduced if there was differential reporting of test results by cases and controls; however, all children presenting to hospital were routinely PCR-tested for SARS-CoV-2 and should be captured by laboratory systems. Adenoviraemia as a cause of non-hepatic acute illness and hospital admission in controls might underestimate the hepatitis association, if also driven by a surge in adenovirus. Finally, while we frequency-matched on key variables, residual confounding could still have occurred.

In conclusion, we found a strong association between adenovirus (41F) viraemia and cases of acute hepatitis of unknown aetiology in the UK outbreak in 2022. Further research is needed on the mechanism of injury and whether adenovirus acts as a trigger with co-factors, such as other viruses and genetic susceptibility, which might interact to contribute to severe disease.

Contributors

SM, CHW, MER, AC, MD, RS, MZ, CW, RBi, AD, and MC contributed to the conception and design of the work. CHa, CHo, CHu, TG, SA, JH, CK, IU-L, AP, GI, DTB, MCO'L, EMD, THD, DH, PB, KR, TR, HC, CG, RBo, AG, SR, and DAK contributed to acquisition of data. RS, GI, CW, CN, MCO'L, CS, FR, SA, AP, NM, CM, LG, EAD, THD, DH, KL, PB, IO, DL, LC, FR, TT, CHa, MG, JC, KH, ER, PS, and CT contributed to processing and analysis (including laboratory) of data. RS, GI, CW, CN, MCO'L, CS, FR, SA, AP, NM, CM, LG, EAD, THD, DH, KL, PB, IO, DL, LC, FR, TT, CHa, CHo, CHu, JC, KH, ER, PS, CT, MZ, SM, CHW, MD, KEB, IU-L, MC, KR, LG, CW, CC, NM, PS, CT, DTB, TR, HC, CG, SR, and DAK had access to the raw data. SM, CHW, AC, MD, RS, KEB, MER, and DL interpreted the data. SM, RS, AC, SNL, MD, and CHW drafted the initial versions of the manuscript. RS, AP, GI, CHW, SM, and AC accessed and verified the underlying data. All authors critically reviewed and edited the manuscript and accept responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

This work is carried out under Regulation 3 of The Health Service (Control of Patient Information; Secretary of State for Health, 2002) using patient identification information without individual patient consent as part of the UK Health Security Agency (UKHSA) legal requirement for public health surveillance. As such, authors cannot make the underlying dataset publicly available for ethical and legal reasons, particularly due to the sensitive information included. However, all the data used for this analysis are included in the manuscript tables and appendix so the analysis can be reproduced. Applications for relevant anonymised data should be submitted to the UKHSA Office for Data Release.

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