



UK Health  
Security  
Agency

# Investigation into acute hepatitis of unknown aetiology in children in England

## Technical briefing 3

19 May 2022

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## Introduction

The UK Health Security Agency (UKHSA) is working with the National Health Service (NHS) and the public health agencies of the 4 nations of the United Kingdom (UK) to investigate the potential cause of an unusually high number of acute hepatitis cases being seen in children in the past few weeks. There is no known association with travel, and hepatitis viruses (A to E) have not been detected in these children.

Unless otherwise stated this technical briefing uses data cut-off of 16 May 2022 to allow time for analyses. More recent case numbers may be cited in other UKHSA public updates but are not included in the analyses presented here. The 4 UK nations are conducting a coordinated investigation and data continues to be reconciled across the UK. For this reason, summary epidemiology data is presented for all 4 nations, whilst some detailed analyses can only be presented for single countries at present.

## Summary

This briefing is produced to share data useful to other public health investigators and academic partners undertaking related work. Although a detailed clinical case review is also taking place, that data is not shared here as, given the small number of cases, there are some risks to confidentiality.

### Cases

As of 16 May 2022, there have been 197 cases of acute non-A-E hepatitis with serum transaminases greater than 500 IU/l identified in children aged under 16 years old in the UK since 1 January 2022. This is the result of an active case finding investigation commencing in April which identified retrospective as well as prospective cases. Eleven cases have received a liver transplant; no cases resident in the UK have died.

New cases continue to be identified across the UK, including cases that meet the case definition and reported cases that are pending classification. Potential reporting lags mean that the rate of new cases is uncertain, though the current rate is more consistent with plateauing than exponential growth. Cases pending classification are usually those in which laboratory testing to rule out known causes of hepatitis has not been completed.

### Working hypotheses

The working hypotheses have been refined. The leading hypotheses remain those which involve adenovirus; however, we continue to investigate the potential role of SARS-CoV-2 and to work on ruling out any toxicological component.

### Associated pathogens

Adenovirus remains the most frequently detected potential pathogen. Amongst 197 UK cases, 170 have been tested for adenovirus of which 116 had adenovirus detected (68%). Amongst cases the adenovirus has primarily been detected in blood. On review of some of the adenovirus negative cases it was notable that some had only been tested on respiratory or faecal samples, and some had been tested on serum or plasma rather than whole blood (whole blood being the optimal sample). It is therefore not possible to definitively rule out adenovirus in cases.

SARS-CoV-2 has been detected in 25 cases of 169 with available results (15%). SARS-CoV-2 serological testing is in process. A range of other possible pathogens have been detected in a low proportion of cases and are of uncertain significance, with some of these under further investigation, including adenovirus-associated virus (AAV) and human herpes virus 6 (HHV6). The inclusive nature of the UKHSA case definition intentionally will pick up some cases of non-A-E hepatitis with recognised causes.

## Adenovirus characterisation

Typing by partial hexon gene sequencing most commonly shows that the adenovirus present in blood is type 41F (27 of 35 cases with an available result, 77%). Whole genome sequencing (WGS) has been attempted on multiple samples from cases but the low viral load in blood samples, and limited clinical material from historic cases, mean that it has not been possible to get a good quality full adenovirus genome from a case as yet.

## Metagenomics

Metagenomics undertaken on blood and liver tissue has detected primarily adeno-associated virus 2 (AAV-2) with certain samples containing reads from Human adenovirus and herpes virus amongst others. One adenovirus 41F (ADV F41) consensus sequence has been generated from a case faecal sample using metagenomics, and when placed in a phylogenetic tree sits with other ADV F41 samples. Additional data from both cases and controls is required to further investigate the role of ADV F41 in these cases.

It is not unusual to detect bystander, reactivating or other incidental species during metagenomic sequencing; however, given the presence of AAV-2 in a number of cases, the significance is being explored through testing of additional sets of controls.

## Toxicology

Toxicological investigations continue and samples have been analysed from all patients for organic compounds, volatile compounds and metals where there were appropriate samples. Paracetamol was detected in some patients and as far as can be ascertained from the information available; its use appears to have been for therapeutic purposes and below toxic levels. Other therapeutics have been detected and among these, fluconazole was investigated more closely and the relationship to therapeutic use and levels are being further assessed. No metals that might be causative was apparent, though some were found to be at higher levels than expected which might be due to pathological change. For organic compounds the current focus is on mycotoxins. Some mycotoxins have been detected in these samples but are not differentially present in cases compared with controls.

## Part 1. Working hypotheses

The following hypotheses are all being actively tested by the investigations in process.

There are increased paediatric acute non-A to E hepatitis presentations due to:

1. A normal adenovirus infection, due to one of:
  - a. Abnormal susceptibility or host response which allows adenovirus infection to progress more frequently to hepatitis (whether direct or immunopathological), for example from lack of exposure during the coronavirus (COVID-19) pandemic.
  - b. An exceptionally large wave of normal adenovirus infections, causing a very rare or under-recognised complication to present more frequently.
  - c. Abnormal susceptibility or host response to adenovirus due to priming by a prior infection with SARS-CoV-2 (including Omicron restricted) or another infection.
  - d. Abnormal susceptibility or host response to adenovirus due to a coinfection with SARS-CoV-2 or another infection.
  - e. Abnormal susceptibility or host response to adenovirus due to a toxin, drug or environmental exposure.
2. A novel variant adenovirus, with or without a contribution from a cofactor as listed above.
3. A post-infectious SARS-CoV-2 syndrome (including an Omicron restricted effect).
4. A drug, toxin or environmental exposure.
5. A novel pathogen either acting alone or as a coinfection.
6. A new variant of SARS-CoV-2.

## Part 2. Update on planned investigations

The investigations include clinical case investigation in the NHS, public health pathogen investigations, and research investigations under the International Severe Acute Respiratory and emerging Infection Consortium (ISARIC) [Clinical Characterisation Protocol](#) with full appropriate consent. ISARIC is funded by UK Research and Innovation and the National Institute for Health Research.

### 2.1 Additional investigations and status

	Investigation	Lead	Status
Analytic epidemiology	Matched case-control study (with residual whole blood samples from hospitalised children for controls) to test association of hepatitis with adenovirus infection	UKHSA	Commenced. Protocol <a href="#">published</a> on UKHSA K-Hub.
	Analysis to investigate co- factors associated with hepatitis in cases	UKHSA	Commenced
	Analysis to investigate factors (demographic and clinical features) associated with severe outcome in cases, stratified by adenovirus infection (case-case study)	UKHSA	Commenced
Surveillance for liver syndromes in children	Enhanced surveillance for severe acute hepatitis in children through British Paediatric Surveillance Unit, and referrals to paediatric liver units	UKHSA	Commenced
Mechanism of liver injury	Investigations on liver tissue to include electron microscopy, further histopathology	NHS	Histopathology review complete; additional investigations planned

	review, T cell subset analysis		
Pathogen investigations	Adenovirus whole genome sequencing from cases and community samples	UKHSA and Great Ormond Street Hospital (GOSH)	Underway with first reports available
	Metagenomic sequencing of blood and liver tissue from cases	UKHSA, GOSH, ISARIC4C (CVR Glasgow)	Underway with first reports available
	Viral culture of adenovirus and phenotypic characterisation including assessment of hepatotropism in vitro	UKHSA and academic partners	Viral cultures of clinical materials negative to date
	Adenovirus and SARS-CoV-2 serology of cases	UKHSA	Testing underway
	SARS-CoV-2 sequencing in positive cases	UKHSA	Reported where available
	Retrospective wastewater analysis for adenovirus	UKHSA	Under consideration
Host characterisation	Harmonised clinical data collation and analysis	ISARIC4C with partners	Recruiting
	Host genetic characterisation	ISARIC4C in partnership with GenOMICC	Recruiting
	Immunological characterisation including T cell activation studies	ISARIC4C with partners	Recruiting
	Transcriptomics	ISARIC4C with partners	Under consideration



## Part 3. Case definitions and summary data for cases in the UK

### 3.1 Case definitions

Case definitions in all UK nations have been revised and harmonised to facilitate clinical reporting. Scotland have changed their case definition to remove possible cases and is moving further towards alignment with England, Wales and Northern Ireland.

#### England, Wales, Northern Ireland case definitions

##### Confirmed

A person presenting since 1 January 2022 with an acute hepatitis which is not due to hepatitis A-E viruses, or an expected presentation of metabolic, inherited or genetic, congenital or mechanical cause [note 1] with serum transaminase greater than 500 IU/L (Aspartate Transaminase-AST or Alanine Transaminase -ALT), who is 10 years old and under.

##### Possible

A person presenting with an acute hepatitis since 1 January 2022 with an acute hepatitis which is not due to hepatitis A-E viruses or an expected presentation of metabolic, inherited/genetic, congenital or mechanical cause [note 1] with serum transaminase greater than 500 IU/L (AST or ALT), who is 11 to 15 years old.

##### Epi-linked [note 2]

A person presenting since 1 January 2022 with an acute hepatitis (non-hepatitis A to E) who is a close contact of a confirmed case.

[note 1] Cases should be reported based on clinical judgement if some hepatitis A-E virus results are awaited, or if there is an acute on chronic hepatic presentation with a metabolic, inherited/genetic, congenital, mechanical or other underlying cause (in Wales, this also excludes known critical illness). If hepatitis A-E serology results are awaited, but other criteria met, these will be classified as 'pending classification'.

[note 2] A person who is epi-linked but also meets the confirmed or possible case definition will be recorded as a confirmed or possible case and their epi-link noted in their record. This prevents double-counting of cases.

#### Scotland case definition

##### Confirmed

A person presenting with a serum transaminase greater than 500 IU/L (AST or ALT) without any known cause [note 3], who is 10 years of age and under or a contact of any age of a confirmed case, since 1 January 2022.

[note 3] Excluding hepatitis A-E, Cytomegalovirus and Epstein-Barr Virus.

## 3.2 Summary data on cases in the UK

As of 16 May 2022, there are 197 confirmed and possible cases in the UK. Of these, 144 cases are resident in England, 26 in Scotland, 15 in Wales and 12 in Northern Ireland (see Figure 1). Between 21 January and 16 May 2022, 11 children in the UK meeting the case definition have required liver transplantation. For the purposes of this summary, all confirmed and possible cases will be referred to as cases. The public health agencies of the 4 nations continue to work with the NHS to classify reported case-patients who have incomplete laboratory information (referred to as 'pending').

## 3.3 Outcomes

Clinical outcomes for 197 cases (at time of clinical notification or interview with parent/guardian) are shown in Table 1. All cases are being followed up for outcome at 28 days after presentation to health services. No cases have died. Of the 197 cases, 180 reported hospitalisation, of whom 11 have required transplantation. It should be noted that the case definitions require high transaminases, and it is possible that there are milder cases which have not been reported.

**Table 1. Outcome status for UK cases on 16 May 2022**

Outcome	Number	Percentage
Died	0	0.0
Not hospitalised	1	0.5
Hospitalisation reported		
Still in hospital	6 [note 1]	3.1
Discharge status not yet ascertained [note 2]	69	35.0
up to 28 days since presentation [note 3]	17	
29 or more days since presentation [note 3]	52	
Discharged or fully recovered	105	53.3
No information	16	8.1
<b>Total cases</b>	<b>197</b>	<b>100</b>

### Notes to table 1

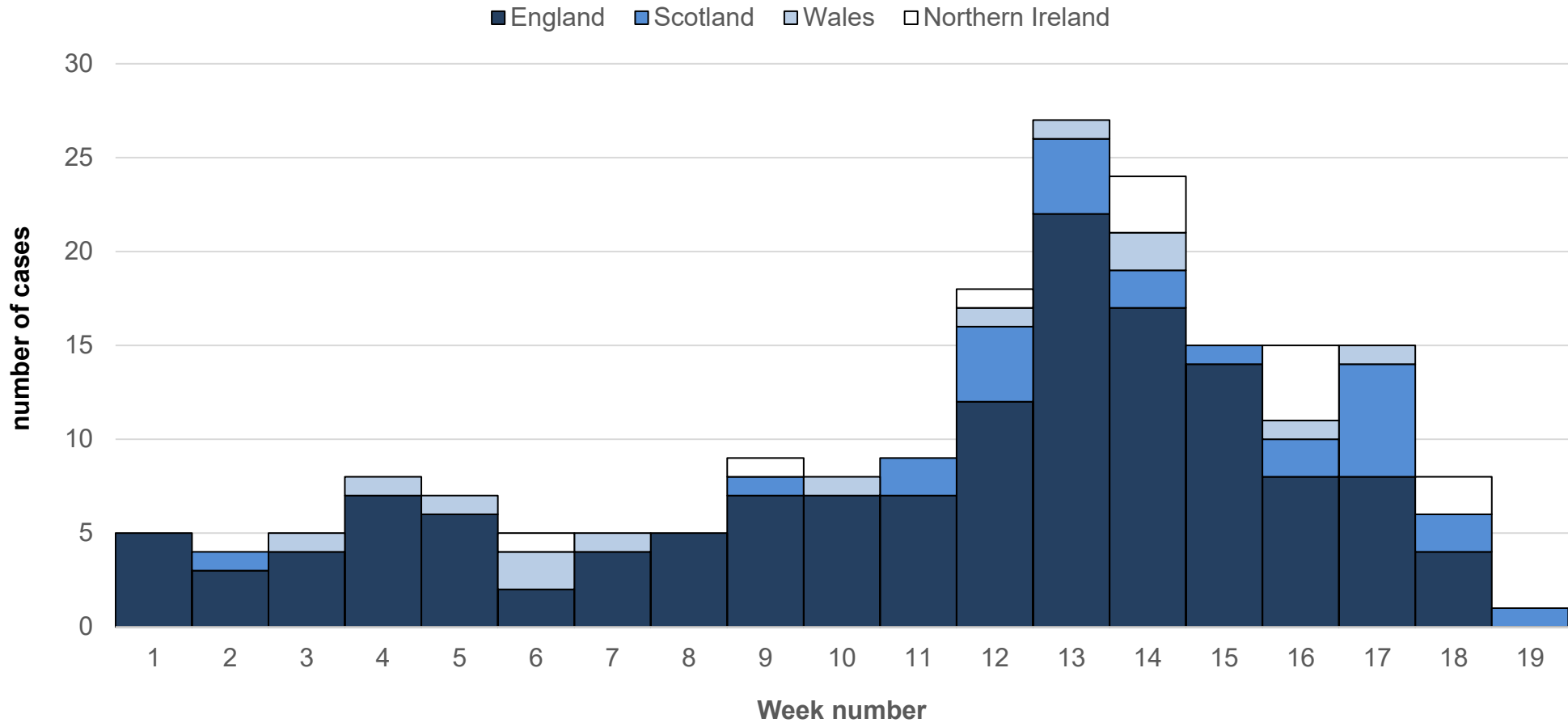
[note 1] available for Scotland only

[note 2] where hospitalised

[note 3] where discharge status is unknown

**Figure 1. Cases by week of presentation, 1 January to 16 May 2022**

Supplementary data is not available for this figure.



Date not available for 2 cases in England and 2 Wales possible cases.

Week is based on hospitalisation date where available, then date of arrival at emergency care department where available, then date of presentation to healthcare.

## 3.4 Possible exposures

Investigations have included interviews of parents conducted by public health specialists to assess a broad range of different exposures (trawling questionnaires), as reported in the [Acute hepatitis: Technical briefing 1](#). In the first 60 case patients in England with data available, no notable features or common exposures were observed in travel, family structure, parental occupation, diet, water source or potential exposures to toxicants, and no association with prior immunosuppression. The public health agencies of Wales, Scotland and Northern Ireland report similar findings through their investigations. Public Health Scotland also report that there are 2 pairs of epidemiologically linked cases.

As detailed in [Technical Briefing 2](#), routine questionnaires found that approximately 70% of children affected, whose families were interviewed, reported recent contact with pet dogs. This finding has been further examined against healthcare data and virological data including adenovirus typing. There is no difference between children with dog contact and those without dog contact in either the patterns of illness or virological detections. As noted in Technical Briefing 2, trawling questionnaires explore many areas of potential exposure, as part of wide-ranging investigations and in identifying areas for more focused investigation. From our extended investigations, there is nothing to indicate a role of dogs in the hepatic syndrome, there are no public health concerns regarding dog contact and the hepatic syndrome. This is no longer an active line of investigation by public health agencies.

Approximately three-quarters of respondents in data for England mentioned paracetamol use. Details of further investigations into paracetamol are included in the toxicological section of this briefing.

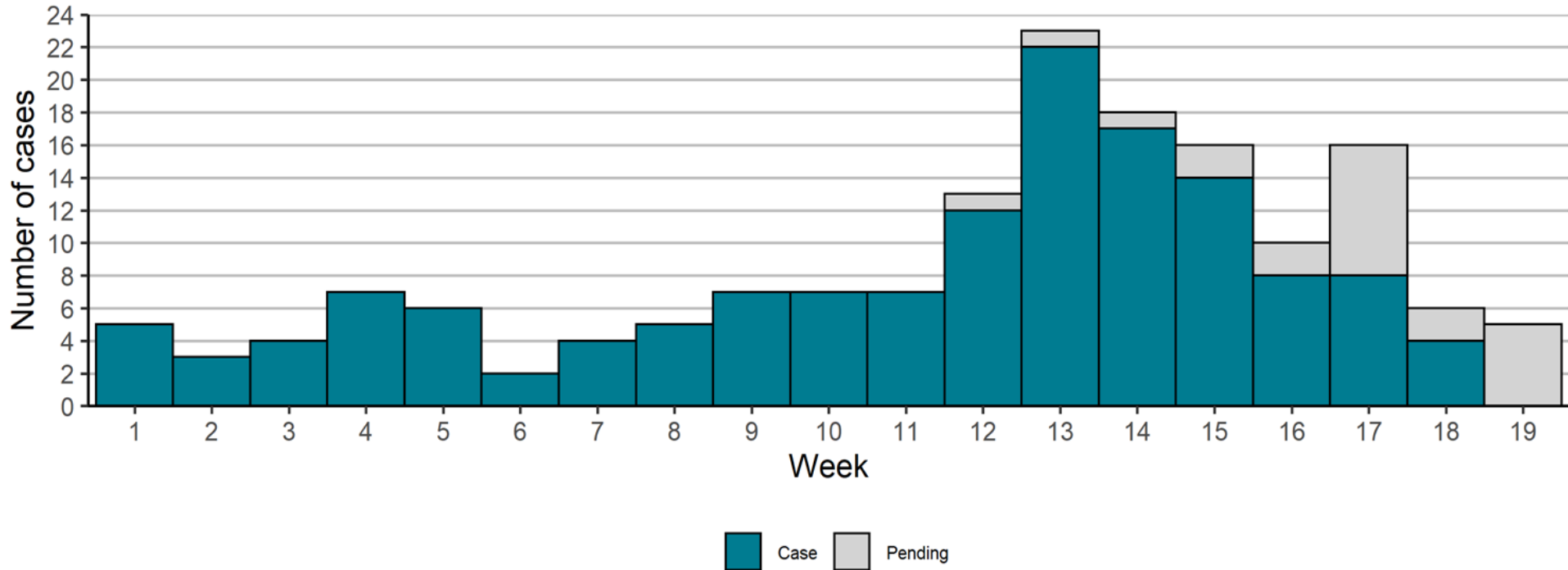
## **Part 4. Cases in England: descriptive epidemiology and clinical data**

### **4.1 Cases in England**

As of 16 May, there were 144 confirmed and possible cases in England. There are no known epidemiologically linked cases in England. Forty-eight potential cases in England are awaiting classification pending further data (see Figure 2). Cases are predominantly aged between 3 and 5 years old (77; 53.5%), median age 3 (interquartile range: 2 to 4 years) and 50% are female. The majority are of white ethnicity (113 out of 131; 86.3%) where information was available.

**Figure 2. Notifications by week of presentation to care and investigation status (Case and pending classification) in England, 1 January to 16 May 2022**

Supplementary data is not available for this figure.



Week is based on hospitalisation date where available, then date of arrival at emergency care department where available, then date of presentation to healthcare. 28 notifications are excluded due to date not being reported. Pending cases are those which have been notified to UKHSA but whose hepatitis A-E serological results are awaited.

## 4.2 Clinical features

The most common presentation reported in cases remains jaundice (99 out of 144; 68.8%) followed by vomiting (83 out of 144; 57.6%). Pale stools were also frequently reported (42.7%). Gastro-intestinal symptoms were commonly reported at presentation including diarrhoea (43.1%), nausea (25.7%) and abdominal pain (36.1%). Additionally, lethargy (48.6%), fever (28.5%) and less frequently, respiratory symptoms (18.1%) were reported. Note that the denominator includes those who have reported the symptom, absence of symptom and unknown (missing information).

## Part 5. Cases: pathogen investigations

### 5.1 Potential pathogens detected through routine clinical testing: all UK cases

Cases have been tested for pathogens at or around the time of admission. Test choice is a local clinical decision and not all cases have been tested for the same pathogens (from [8 April 2022](#), UKHSA recommended a panel of tests to perform on all cases). All hospital admissions would have SARS-CoV-2 tests. Testing information is gathered from a variety of sources, including direct reports from clinicians, direct reports from laboratories, information from UKHSA reference laboratories and from linkage to data sources including Second Generation Surveillance System (SGSS) and the COVID-19 Unified Sample Dataset.

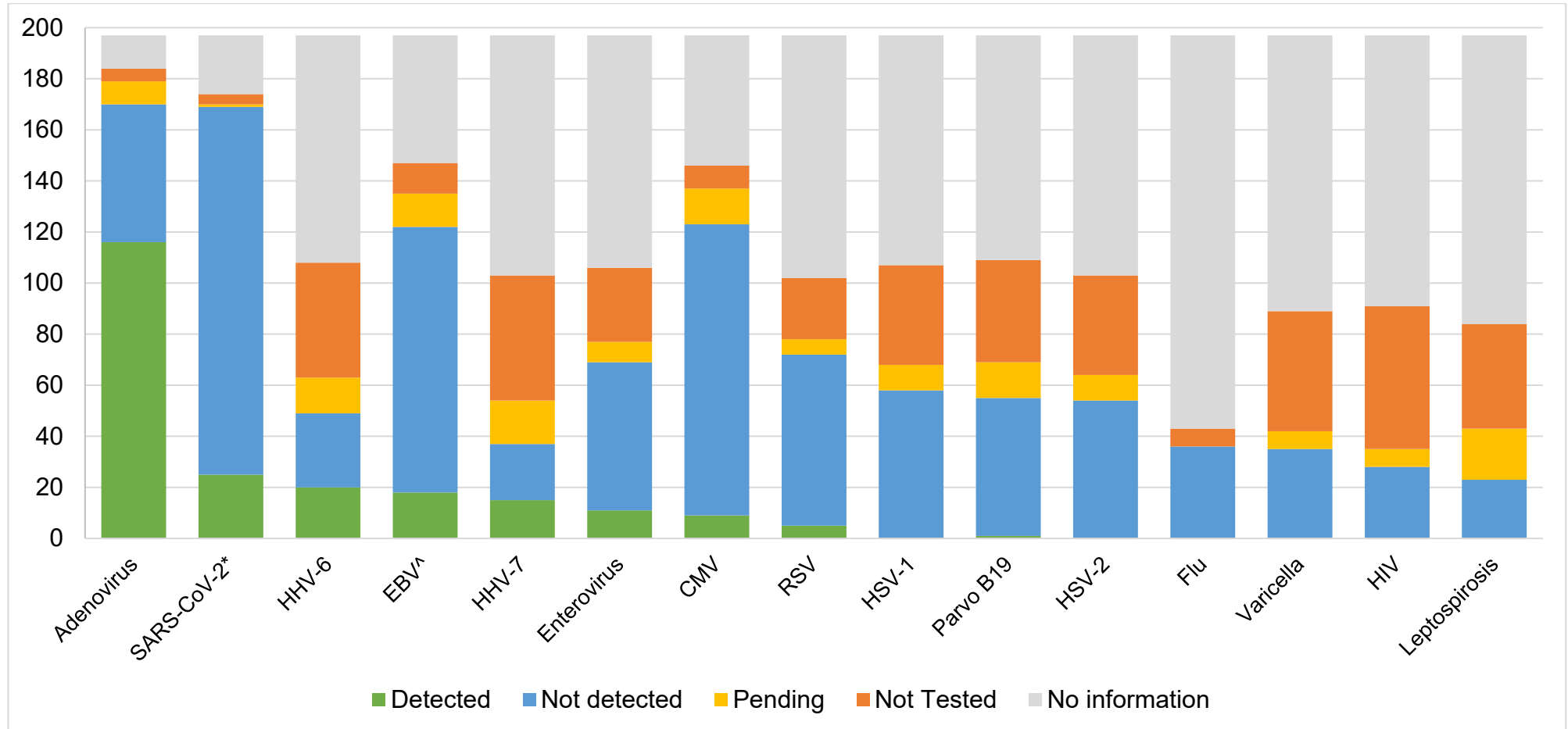
Adenovirus remains the most common pathogen detected (Figure 3). Of 197 UK cases, 179 were tested for adenovirus. Adenovirus was detected in 116 (68%), and not detected in 54 cases. Nine cases have results pending, 5 were not tested, and no information is available for 13 cases. On a review of some of the cases tested for adenovirus which were negative, it was apparent that several had not been tested in whole blood (the preferred sample type), and additional retrospective testing is being explored.

A more detailed breakdown of pathogen results for cases, where available to UKHSA, is provided in Figure 3.



**Figure 3. Pathogens tested for and results in cases in UK**

Supplementary data is not available for this figure.



\*SARS-CoV2 testing presented here is based on testing around hospital admission or attendance (had previously included up to 2 weeks prior for England cases).

^ including one past positive.

## 5.2 Adenovirus

Adenovirus was the most common pathogen detected.

In England adenovirus was detected in 91 of 122 cases where results are available (75%). Further analysis below relates to cases from England. Further details on adenovirus testing can be found in [Technical Briefing 2](#).

For cases in England, by sample type based on the data reported, adenovirus was detected more commonly in blood or serum samples from cases (77 out of 97; 79.4%), than in stool (43.9%) or respiratory (27.3%) samples; however, a consistent sample set has not been tested for most cases (see Table 2). The detection of enteric adenoviruses in blood is so far not accompanied by detection of enteric adenovirus in stool at the same time, but in a handful of cases an enteric adenovirus has been found in blood, with a different adenovirus found in faecal or respiratory materials, indicating a possible mixed infection.

Of the 31 cases where adenovirus was not detected, 13 had no testing on blood reported, which appears to be the most relevant sample type for the syndrome, and there are potential performance differences between assays in clinical use. Therefore, the presence of adenovirus cannot be definitively excluded in sample types other than blood when it is not detected.

Thirty-five of the 77 cases with adenovirus in blood have been successfully subtyped, of which 27 (77%) are type 41F.

Of the 8 England-resident patients who required a liver transplant, 7 were tested for adenovirus in blood samples and the virus was detected in all 7. Five were successfully subtyped, as 41F.

**Table 2. Adenovirus testing [note 1] results of cases resident in England**

Adenovirus testing	Number of cases	Number of cases with each sample type (there may be multiple samples per case)			
		Blood	Stool	Respiratory	Other or unknown
Positive	91 (74.6%)	77	18	12 (27.3%)	17 (73.9%)
(% positivity, excluding pending)		(79.4%)	(43.9%)		
Negative	31	20	23	32	6
Pending	9	6	12	7	13
<b>Total tested</b>	131	103	53	51	36

### Notes on table 2

[note 1] testing locations: any diagnostic laboratory.

## Sample analysis

The summary of findings as of 13 May 2022, in different sample types is based on samples submitted (real world data). There is considerable variation in samples that have been submitted for each case. Each sample has been tested using 2 different PCR targets, Hexon gene PCR and Fibre gene PCR. Hexon gene PCR target is a pan adeno detection system whereas the Fibre gene detects enteric adenoviruses 40 and 41. PCR detection tests are based on published studies<sup>1</sup>.

Using the dual PCR target approach, there is good concordance for detection of adenovirus in blood using both targets, showing a similar distribution of cycle threshold (Ct) values for detection. Cycle threshold (Ct) values are based on the number of cycles conducted before detecting the virus on a PCR test. The fewer cycles needed to detect the virus, the more virus there is in the sample (and therefore higher viral load).

**Table 3. Comparison of different PCR targets in blood samples (whole blood, serum, plasma)**

PCR outcome of blood samples from cases		Hexon gene PCR		Total
		Positive	Negative	
Fibre gene PCR	Positive	54	4	58
	Negative	11	35	46
Total		65	39	104

<sup>1</sup> Pan Adeno: Heim A, Ebnet C, Harste G, Pring-Åkerblom P. 'Rapid and quantitative detection of human adenovirus DNA by real-time PCR'. Journal of Medical Virology 2003: volume 70, pages 228 to 39  
Enteric Specific: Tiemessen CT, Nel MJ. 'Detection and typing of subgroup F adenoviruses using the polymerase chain reaction'. Journal of Virological Methods 1996 May: volume 59, issues 1 to 2, pages 73 to 82. doi: 10.1016/0166-0934(96)02015-0. PMID: 8793832

Figure 4. Ct value comparison of blood sample from cases – Hexon versus Fibre gene PCR (n=54)

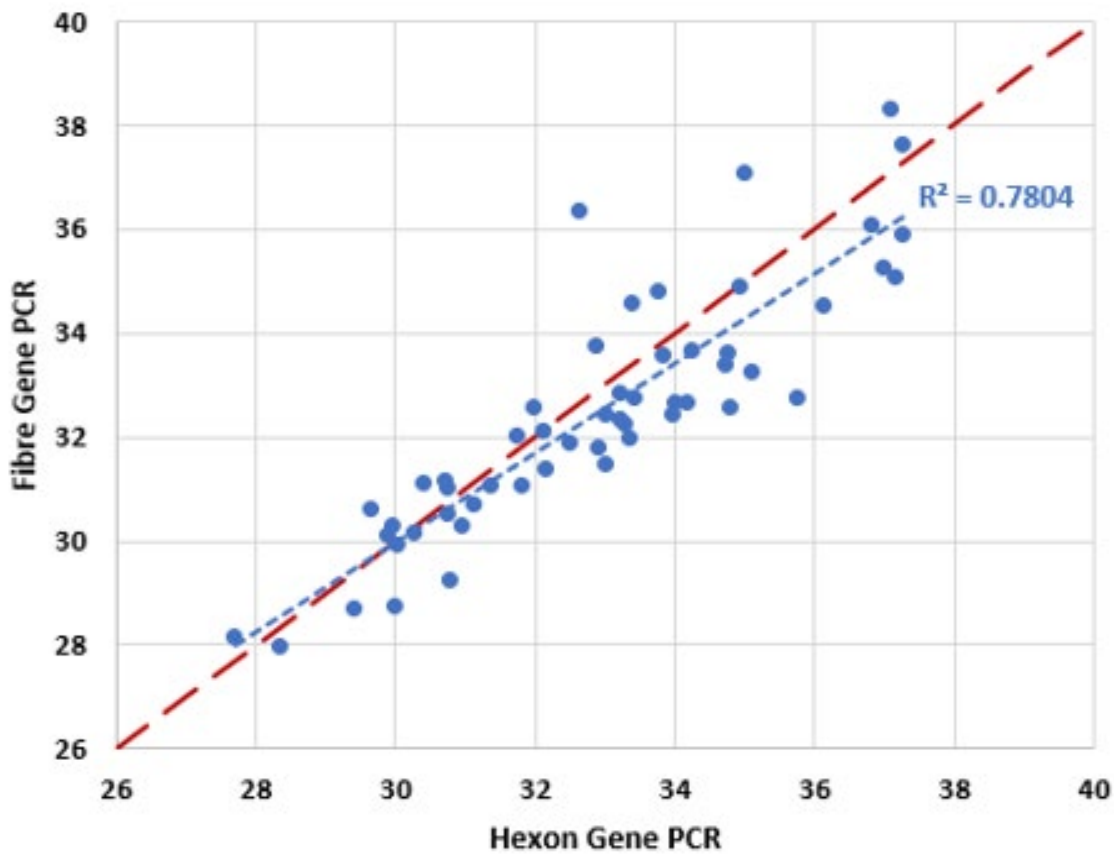
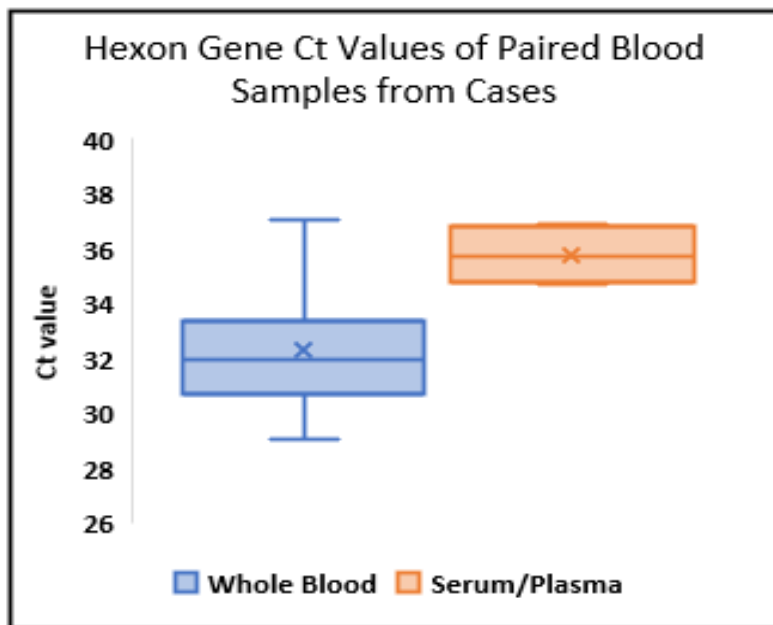


Figure 5. Comparison of different sample types using Hexon Gene PCR. Detection in cases (N=24)



**Table 4. Comparison of different sample types using Hexon Gene PCR. Detection in cases (N=24)**

Cases with paired samples N=24	Whole blood	Serum/plasma
PCR detection (%)	23 out of 24 (96%)	4 out of 24 (17%)
Range	29.06 to 37.90	34.72 to 36.90
Mean	32.3	35.6

**Table 5. Comparison of detection in Blood vs faecal material from 22 cases with paired samples**

Cases with paired blood and faecal samples	Blood (any sample) (+)	Blood (-)	Total
Faecal (+)	6 [note 1]	2 [note 2]	8 [note 3]
Faecal (-)	12	2	2
Total	18	4	22

**Notes to Table 5**

All adenovirus found in faecal material is type C adenovirus

[note 1] Evidence of mixed infection in 6 children, sequence confirmed

[note 2] 1x1c 1x2c

[note 3] 2x 2c, 2x1c, 2x 5c

All adenovirus found in blood is Ad 41

Partial genome sequencing based on Hexon gene

**Table 6. Comparison of detection in Blood vs respiratory material from 11 cases with paired samples**

Cases with paired blood and respiratory samples	Blood (+) any sample	Blood (-)	Total
Respiratory (+)	3 [note 1]	2	5
Respiratory (-)	5 [note 2]	1	6
Total	8	3	11

**Notes to Table 6**

Sequence confirmed type not available for all samples due to low viral load

[note 1] 1x 2c for both respiratory and blood detection

[note 2] All detections sequence confirmed Ad 41

Partial genome sequencing based on Hexon gene

Figure 5 indicates that whole blood is a better matrix for detection of adenovirus than serum/plasma and the use of serum or plasma alone for diagnostic detection will miss the detection of an adenovirus in the majority of cases (with the caveat that the paired samples received may not have been identical in timing). Not all samples have yielded sufficient material

for genotyping through partial genome sequence analysis. There is evidence of mixed infections in several cases involving several different subtypes. Adenovirus 41 has not so far been found in faecal samples from cases, even where the case has detectable adenovirus 41 in blood.

## 5.3 SARS-CoV-2

For cases resident in England, 13 cases tested positive for SARS-CoV-2 on admission (PCR or lateral flow device), of 123 cases with available test data (10.6%). An additional 3 cases tested positive in the 8 weeks prior to admission (1 of whom was negative on admission) giving a total period prevalence of 16 out of 125; 12.8%. Weekly SARS-CoV-2 positivity in the hepatitis case cohort is broadly consistent with NHS pillar 1 COVID-19 testing and the ONS infection survey prevalence; more focused investigations are required to determine any role of SARS-CoV-2 in the hepatic syndrome. Serological testing of cases is in process to explore prior infection further, however, the high population cumulative prevalence of SARS-CoV-2 will make the interpretation of this data challenging. Seven cases were co-infected with adenovirus and SARS-CoV-2.

## 5.4. Metagenomic sequencing

### Blood and liver analyses

Metagenomics data from 28 UK samples, of which 10 samples (9 cases) sequenced at GOSH, and 18 samples (9 cases) from Medical Research Council-University of Glasgow Centre for Virus Research (MRC-CVR) have been analysed. Sample types include 8 liver samples, 7 faecal, 6 blood, one rectal, and 6 throat.

Analysis of metagenomics data from blood and liver samples (n=14) has been initially performed using Kraken2. One sample contained insufficient viral reads for further analysis. Viral reads in the remaining 13 samples show adeno-associated dependoparvovirus A in all samples. Four samples contain reads mapping to human adenovirus, which includes viruses such as Adenovirus 41F. All samples also contain reads identifying as Human Herpes Virus, including viruses such as HHV6. Work is ongoing to determine sub-types. Four of 8 liver samples also contain Human Polyomavirus. The classifier used in initial analysis does not have the specificity required to identify species and subspecies. Work is ongoing.

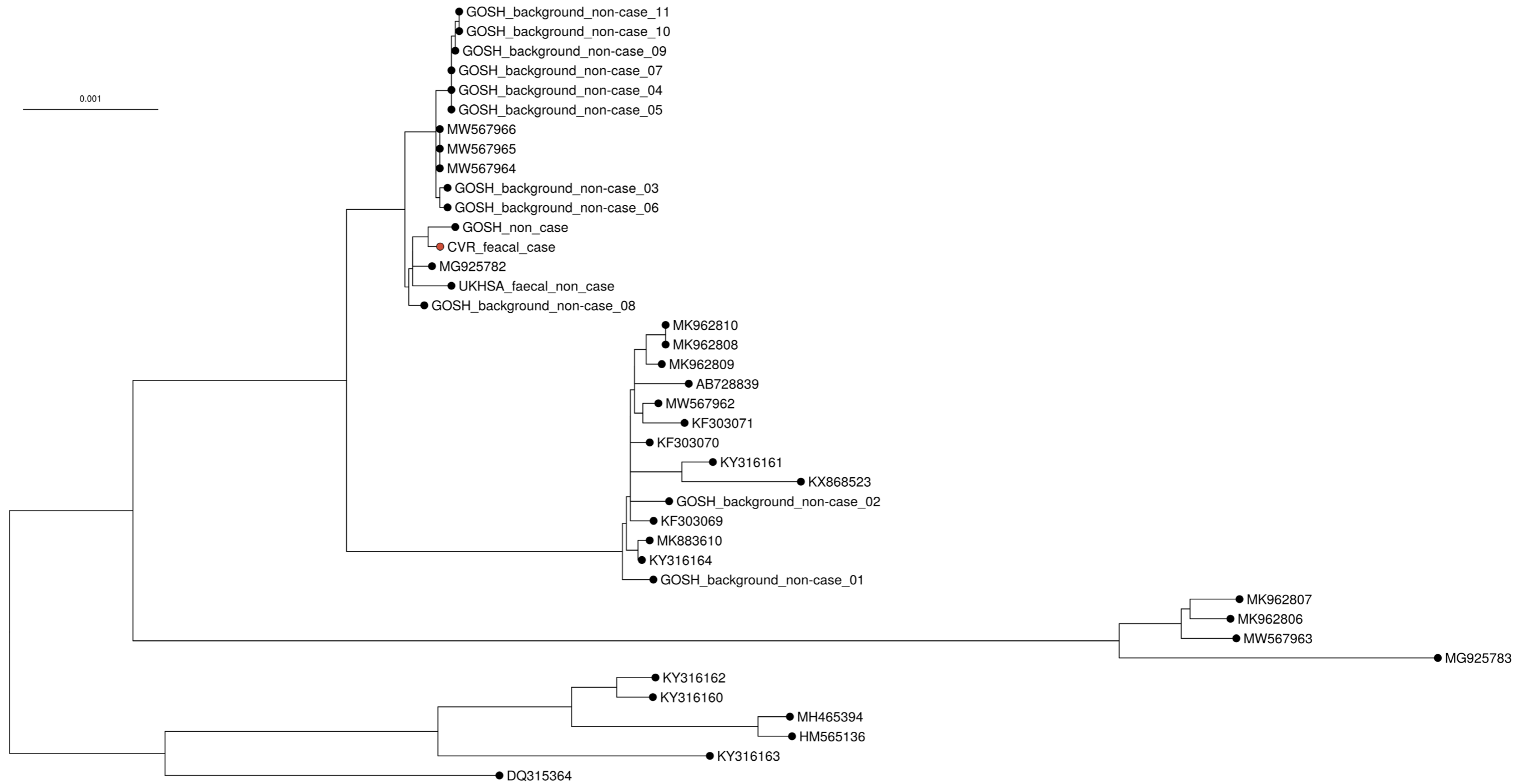
It was previously reported that adeno-associated virus 2 (AAV2) was detected in both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) fractions through metagenomic analysis, in samples from cases in England and Scotland. Analysis is ongoing.

One adenovirus 41F consensus sequence from a case faecal sample has been generated from metagenomic data by MRC-CVR. This sample contains 3 single nucleotide polymorphisms (SNPs) not present in ADV 41F background sequences from Genbank. Analysis of blood and

liver metagenomic samples identified as containing ADV 41F reads was performed, but sequence data were of insufficient quality to generate consensus sequences for comparison. Further WGS of ADV 41F is required. A tree containing the MRC-CVR faecal sample alongside background samples is shown in Figure 6. The tree contains currently unpublished GOSH samples, as well as a non-case recent WGS faecal sample.

Academic and clinical centres which have or can generate adenovirus WGS data are asked to share consensus genomes to an International Nucleotide Sequence Database Collaboration such as GenBank to assist characterisation of circulating adenovirus strains internationally.

**Figure 6. Whole genome phylogenetic tree containing ADV 41F consensus sequence generated from metagenomics of faecal sample by MCR-CVR. Background ADV 41F sequences gathered from GenBank, and GOSH. This tree has been rooted using ADV C genome sequences from GenBank not shown in this image. Case sequences are shown in red, background sequences shown in black. Supplementary data is not available for this figure.**





## Part 6. Cases: Toxicology investigations

As of 17 May 2022, 222 samples have been received from 145 confirmed cases and 77 age-matched controls from the biobank. Of these, 170 are serum or plasma, 29 urine and 23 whole blood. The 145 confirmed case samples are from 44 patients, reflecting multiple time-point samples from several patients. Of the samples from confirmed cases, 69 were obtained within 5 days of presentation or hospital admission, 27 within 5-10 days and 26 were from more than 10 days after presentation or admission. The remainder cannot currently be staged due to missing information. Sera from 41 confirmed cases and 28 controls have been analysed by Liquid Chromatography/High Resolution Mass Spectrometry (LC/HRMS) in all modes (see below), 28 cases and 23 controls by Gas Chromatography/Mass Spectrometry (GC/MS) and 24 cases and 1 control by Inductively Coupled Plasma Mass Spectrometry (ICPMS) for metals. The ICPMS analysis is initially quantitative and so not as dependent on a control comparison as the LC/MS and GC/MS.

LC/HRMS targets substances with different chemistries: polar and nonpolar, each with positive and negative ionisation for organic molecules and metabolites. GC/MS is being used for volatile and semi-volatile organics and Inductively Coupled Plasma Mass Spectrometry (ICPMS) for metals/elemental analyses.

The LC/HRMS gives a qualitative report of the masses of chemicals detected in the samples, where there is a sufficiently strong signal. These masses and fragmentation patterns are compared against a variety of databases to derive possible substance identification. Differential observed response for each mass is compared between controls and cases to determine potential substances of interest. Upon identification potential causative substances identified can then be compared to a reference standard, if this is available, for confirmation of identity and quantitative assessment. The first samples obtained were from late-stage cases and yielded hundreds of organic substances consistent across patient samples, but many (such as bile acids, bile salts and therapeutics) are related to the pathophysiology or management of acute hepatitis. Several therapeutic moieties including antibiotics, ursodeoxycholic acid, vitamins, paracetamol and fluconazole have been detected. Some of these have been reported as being administered in hospital as part of case management. Paracetamol and fluconazole have been investigated in more detail because of their known relationship with liver injury dependent on dose and individual susceptibility.

### Paracetamol

The levels of paracetamol have been quantitated, with plasma and urine levels in the collected samples and in early samples below those at which liver injury would be expected. Additionally, most strongly positive samples are late stage, indicating use in hospital (confirmed for some cases). It therefore appears highly unlikely that paracetamol is causative, with one caveat that

the very low levels (below limits of quantitation) detected in the early samples could reflect dosing several days prior to sample collection, when most paracetamol will have been excreted prior to presentation. To fully discount paracetamol as causative, it is necessary to know when paracetamol was used prior to sample collection and at what dose. This information is being sought.

## Fluconazole

This agent has been associated with drug-induced liver injury in a small percentage of individuals, reflecting individual susceptibilities. This compound has been detected in 4 early samples and one late sample. It has not been quantitated but was easily detected and identified. Detection likely reflects use on or near admission and therefore is unlikely to be causative but as with paracetamol more information on therapeutic use (time/dose) is being sought.

## Mycotoxins

Mycotoxins are known to cause liver injury with a pathology like that seen in these patients. Some mycotoxins have been detected in these samples but are not differentially present in cases compared with controls. It is likely though that most that may be causative in these cases will have been excreted prior to or soon after presentation. Nevertheless, standards and other necessary materials are currently being procured to set up the analysis method and mycotoxins will be the focus of work moving forward.

## Part 7. Relevant surveillance data

### 7.1 Increases in laboratory reports of new diagnoses and exceedances in potentially relevant pathogens

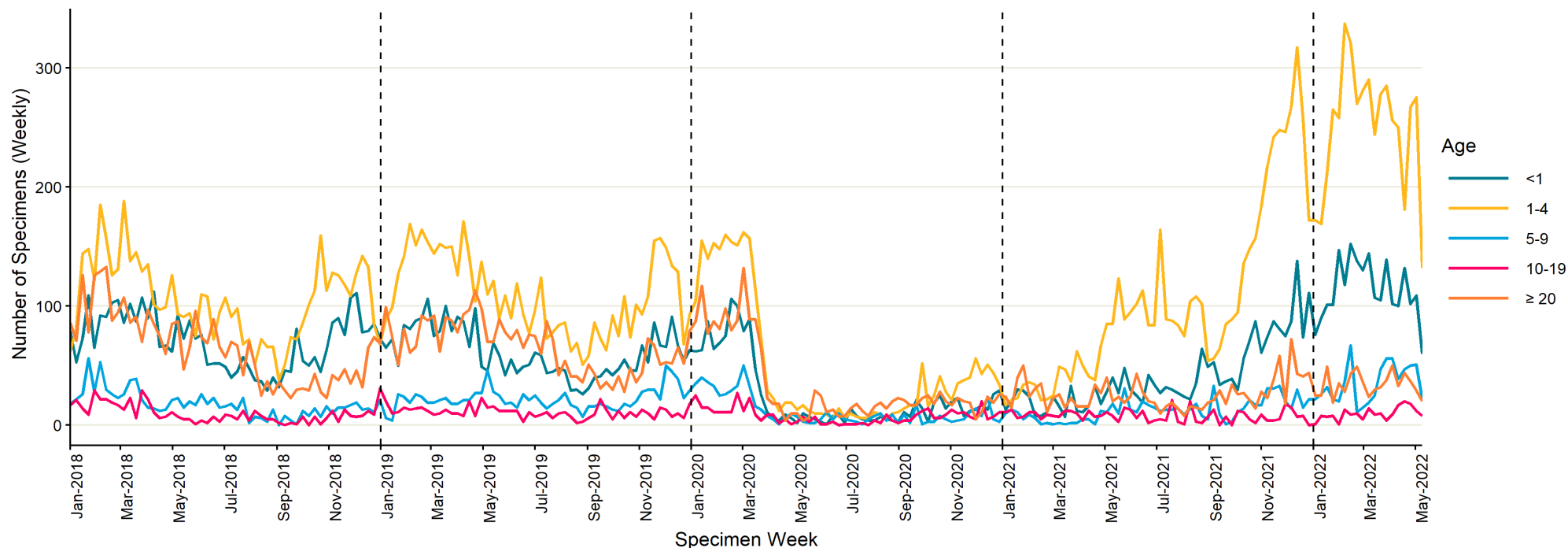
Laboratory data on pathogens reported by the NHS and public health laboratories through SGSS are routinely monitored for changes in trends and statistical exceedances. Increases in new laboratory diagnoses and statistical exceedances have been observed in adenovirus, enterovirus, human metapneumovirus, rhinovirus and norovirus in under 10 year olds since the end of 2021. There has been a marked exceedance of adenovirus, apparently driven by adenovirus in faecal samples and in the 1 to 4 year old age group although the number of laboratory diagnoses appear to be decreasing now. However, it is too soon to comment on any trends as random fluctuations occur and recent school holidays may have temporarily reduced social mixing.

#### Adenovirus reports from diagnostic laboratories

Adenovirus positive tests from routine clinical testing are recorded in SGSS and can be analysed by UKHSA. The testing patterns for adenovirus are likely to be variable, influenced by clinical presentation, and as it is not a notifiable disease there is also likely to be under-reporting. Negative results are not recorded in SGSS, but a different system (Respiratory Datamart) does take positive and negative test data from a sentinel lab network only, but these are respiratory samples. Some laboratories have been asked to provide data on positive and negative faecal and blood samples to understand background adenovirus positivity. These data are being collated but initial data from Micropathology Ltd that provides testing for several NHS trusts across England indicate positivity of 10% for blood and 18% for faecal samples in children 10 years and under (excluding known cases) during March and April 2022.

Reports in England of positive adenovirus tests from any site in 1 to 4 year olds are higher compared to the previous 5 years (see Figure 7). Between November 2021 to April 2022, approximately 200 to 300 specimens of adenovirus were reported into SGSS per week compared to 50 to 150 per week in the pre-pandemic period and less than 50 per week between March 2020 and May 2021. The increase in younger age groups begins in November 2021. This pattern is also seen specifically in enteric samples in the same age group (see Figure 8). There have been some declines in enteric adenovirus detections in recent weeks, though very recent data may reflect incomplete reporting.

**Figure 7. Adenovirus positive specimens (any type) by age and week of specimen, England 1 January 2018 to 15 May 2022 [note 1]**  
Supplementary data is not available for this figure.

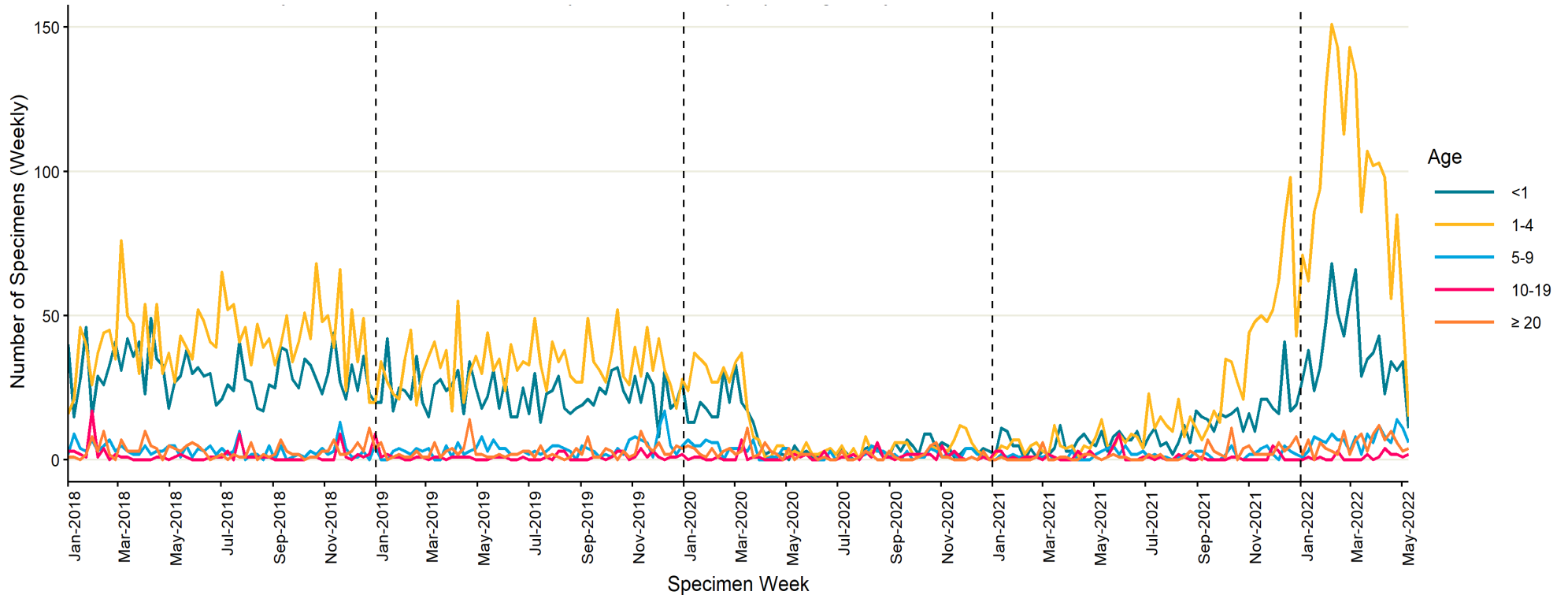


Data source: SGSS

[note 1] Dotted lines indicate year start, most recent point affected by reporting delay. The presented figures are based on laboratory reports through SGSS. Testing and reporting procedures vary by virus, UKHSA centre and over time, including short-term trends in testing. Therefore, comparisons should be done with caution.

**Figure 8. Adenovirus positive faecal/gastro-intestinal specimens by age and week of specimen, England 1 January 2018 to 15 May 2022 [note 1]**

Supplementary data is not available for this figure.



Data source: SGSS

[note 1] Dotted lines indicate year start, most recent point affected by reporting delay. The presented figures are based on laboratory reports through SGSS. Testing and reporting procedures vary by virus, UKHSA centre and over time, including short-term trends in testing. Therefore, comparisons should be done with caution.

## Exceedance monitoring

There has been an exceedance from end of 2021 in adenovirus from all sites - faecal and respiratory - in younger children but not among older children or adults. Methodology for exceedance monitoring can be found in the [Sources and acknowledgements](#) section.

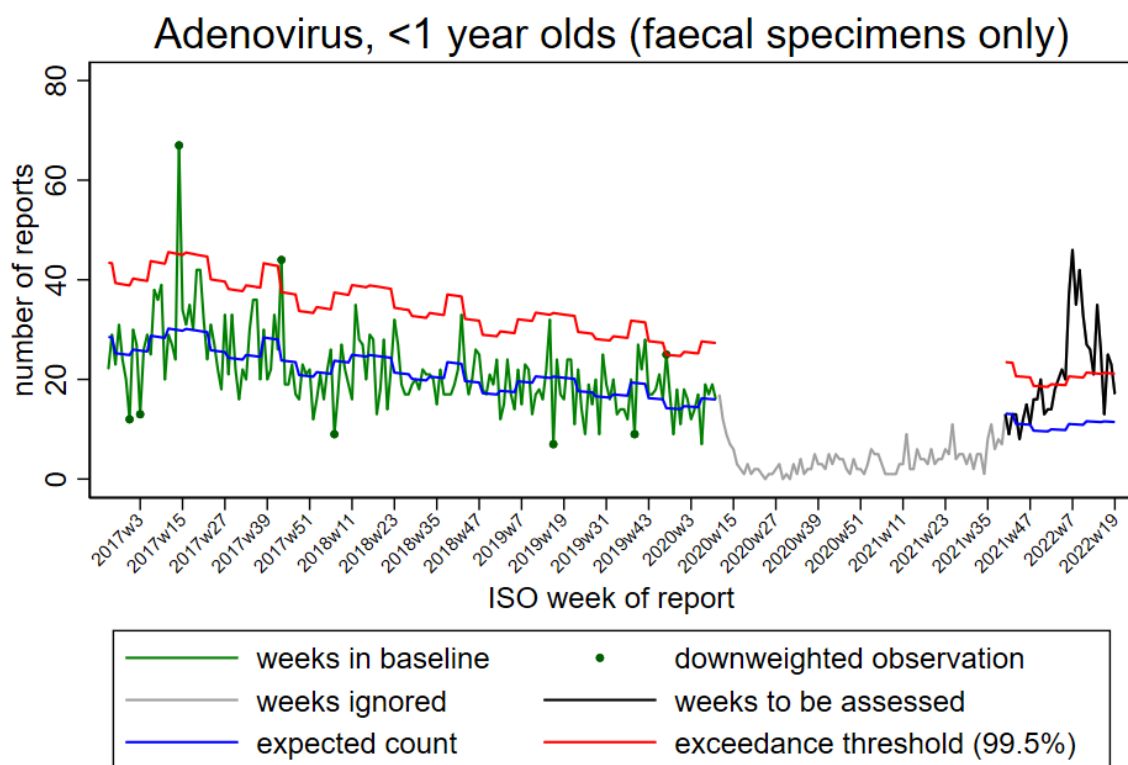
The plots in Figure 9 show trends in adenovirus reports in children from faecal samples. The graphs compare recent data to the trends seen in previous years. Values above the red line indicate periods where the figures are higher than would be expected within the normal range.

### Faecal specimens adenovirus exceedance

Exceedance for faecal specimen adenovirus was seen in all younger children under-1 year to 9- years old. Although laboratory positive reports have declined in recent weeks, the number for children aged 3 to 5 years remains above the exceedance threshold. Exceedance for faecal specimen adenovirus was not observed in older children or adults.

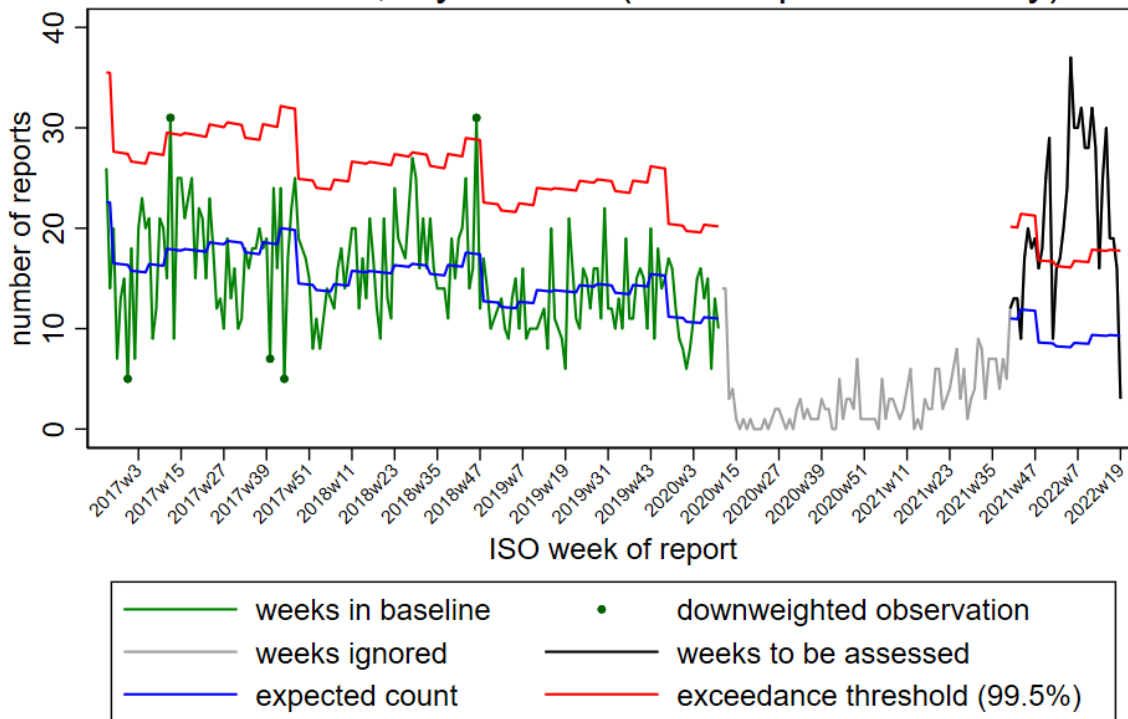
**Figure 9. Exceedance for faecal specimen adenovirus as seen in under 1 year to 9 years old**

Supplementary data is not available for these figures.



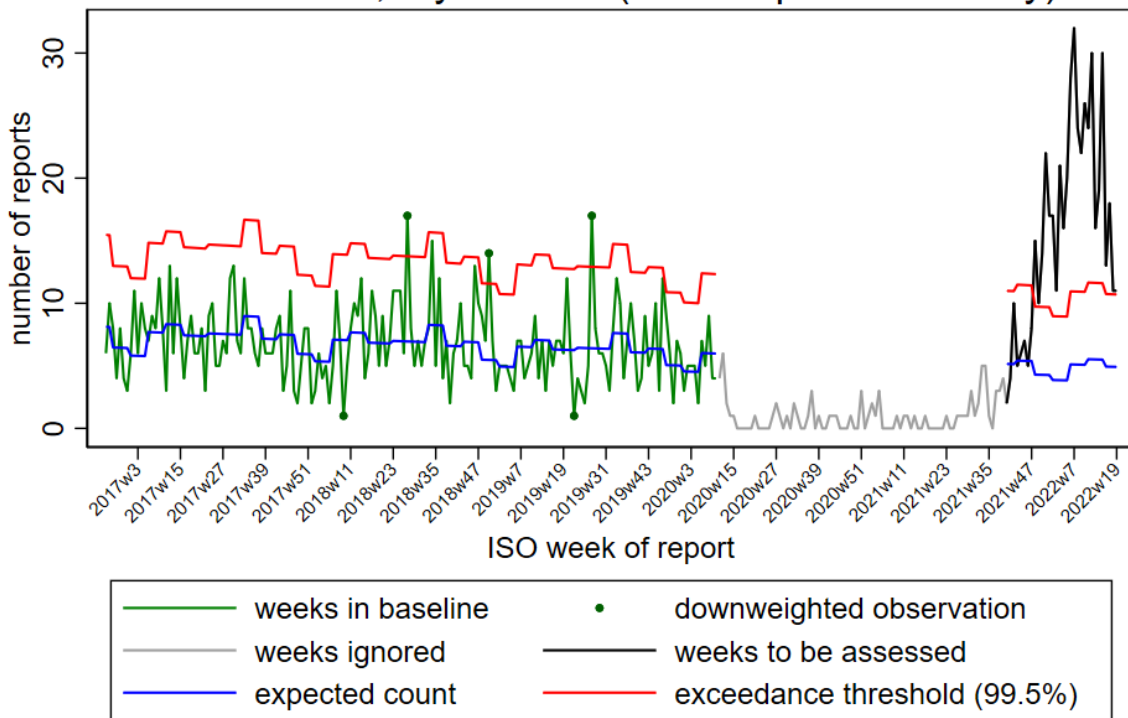
Data source : SGSS vw\_Weekly\_Exceedance table, latest week of reporting 18, 2022

### Adenovirus, 1 year olds (faecal specimens only)



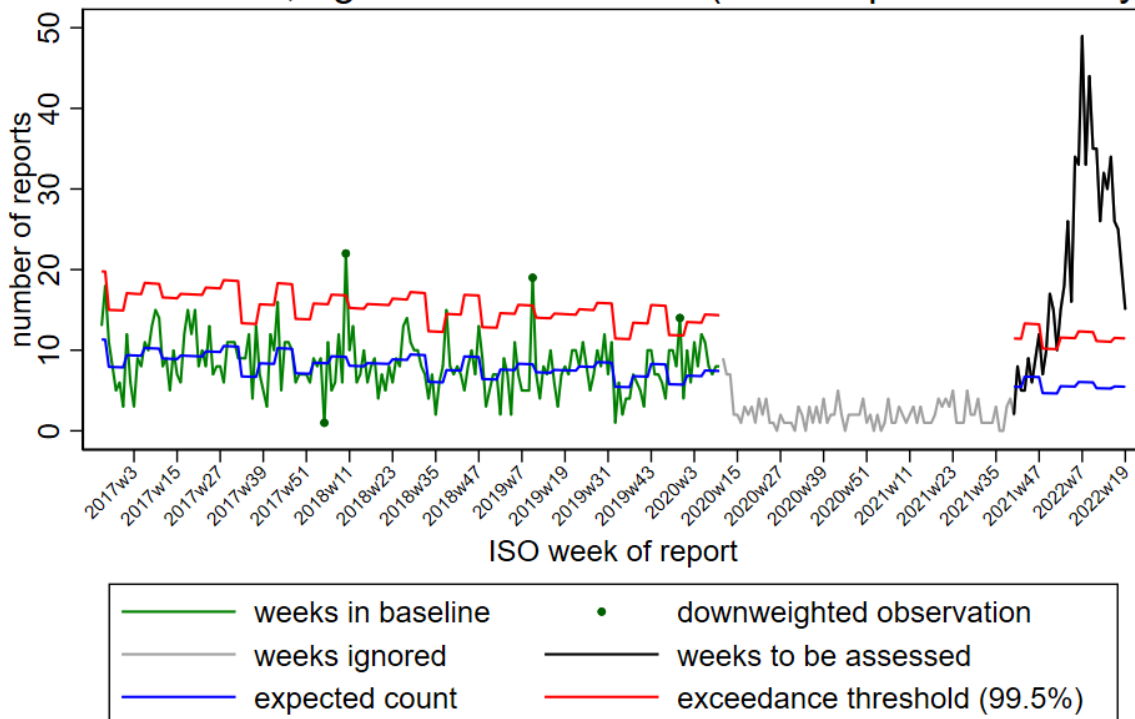
Data source : SGSS vw\_Weekly\_Exceedance table, latest week of reporting 18, 2022

### Adenovirus, 2 year olds (faecal specimens only)



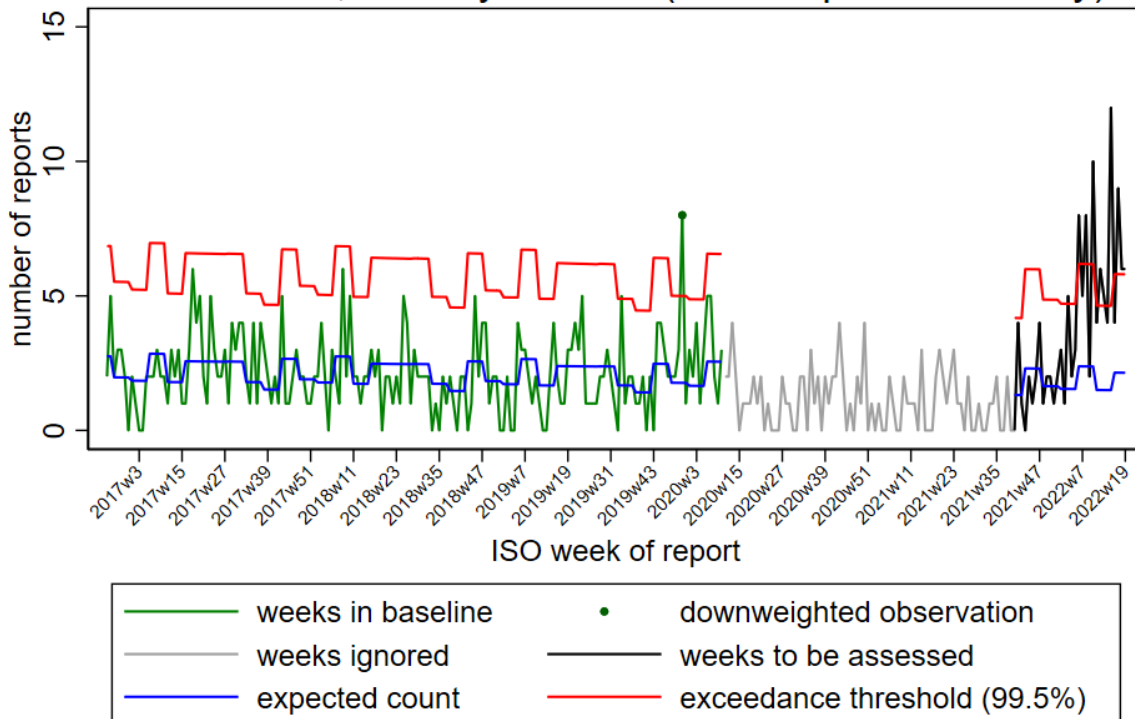
Data source : SGSS vw\_Weekly\_Exceedance table, latest week of reporting 18, 2022

### Adenovirus, aged 3 to 5 inclusive (faecal specimens only)



Data source : SGSS vw\_Weekly\_Exceedance table, latest week of reporting 18, 2022

### Adenovirus, 5 to 9 year olds (faecal specimens only)



Data source : SGSS vw\_Weekly\_Exceedance table, latest week of reporting 18, 2022



# Sources and acknowledgments

## Data sources and methodologies

### Exceedance monitoring

UKHSA monitors trends in pathogens through routine and ad hoc surveillance of laboratory notifications of positive test results undertaken as part of clinical care reported through SGSS. Exceedance monitoring is also used as part of assessing whether disease activity is above that expected. This uses a statistical threshold based on a moving average and secular trends in detection of a pathogen (thus addressing both changes in laboratory testing practices over time, and seasonal variation in disease activity). Further details of the statistical methods are described by [Noufaily and colleagues](#).

### Data sources

Data used in this investigation is derived from:

Second Generation Surveillance System (UKHSA)  
Secondary Uses Service (NHS Digital)  
Emergency Care Data Set (NHS Digital)  
Admitted Patient Care (NHS Digital)  
Respiratory Datamart (UKHSA)  
Syndromic surveillance (UKHSA)  
NHS Blood and Transplant  
COVID Unified Data Set (UKHSA)  
NOIDs (UKHSA)  
HPZone (UKHSA)  
International Severe Acute Respiratory and emerging Infection Consortium (ISARIC) UK  
ONS Coronavirus Infection Survey  
All Wales Laboratory Information System and Datastore  
Health Protection Case and Incident Management System Wales  
MRC-University of Glasgow CVR  
Clinical Team at NHS Lothian  
Clinical Team at NHS Greater Glasgow and Clyde

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UKHSA Epicell

UKHSA Genomics Public Health Analysis

UKHSA Toxicology Cell

UKHSA Virology Cell

UKHSA Virus Reference Department

ISARIC

Glasgow Centre for Virus Research

Great Ormond Street Hospital with University College London

Public Health Scotland

Public Health Wales

Public Health Agency Northern Ireland

# About the UK Health Security Agency

UKHSA is responsible for protecting every member of every community from the impact of infectious diseases, chemical, biological, radiological and nuclear incidents and other health threats. We provide intellectual, scientific and operational leadership at national and local level, as well as on the global stage, to make the nation health secure.

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