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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Mandal S, Simmons R, Ireland G, et al. Paediatric acute hepatitis of unknown aetiology: a national investigation and adenoviraemia case-control study in the UK. *Lancet Child Adolesc Health* 2023; published online Sept 26. https://doi.org/10.1016/S2352-4642(23)00215-8.

SUPPLEMENTARY APPENDIX

Paediatric acute hepatitis of unknown aetiology: national investigation and adenoviremia case-control study, United Kingdom

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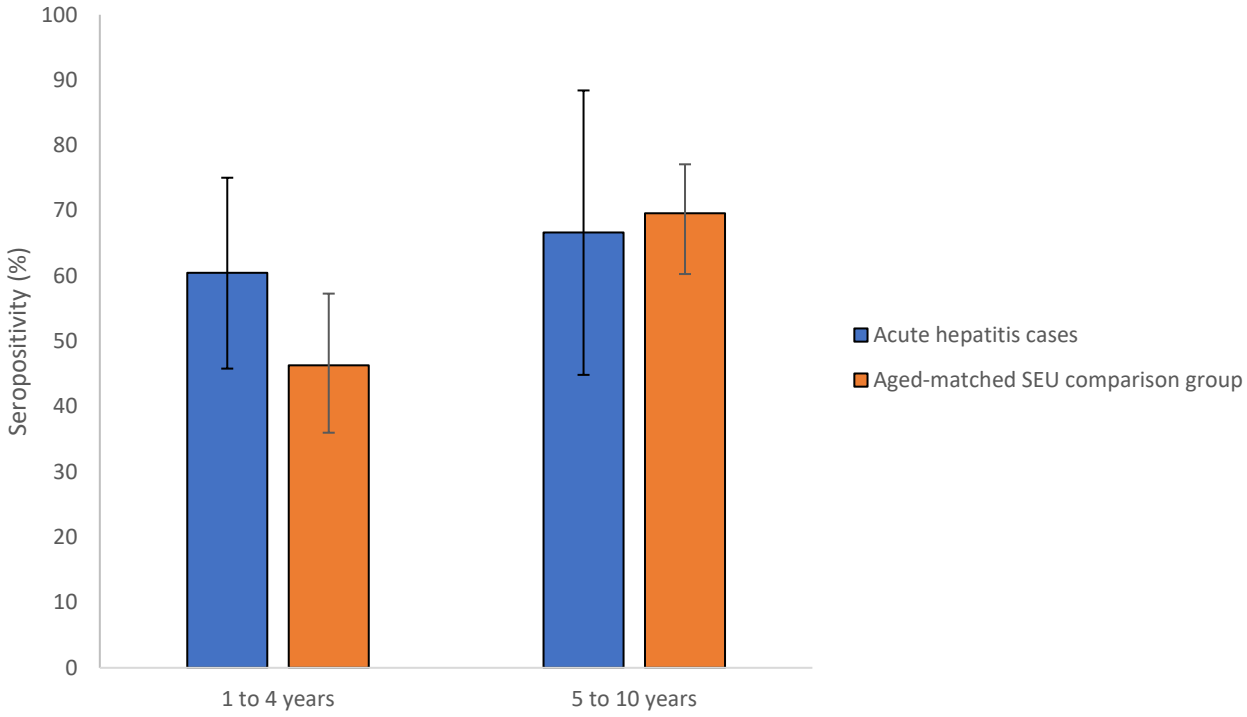
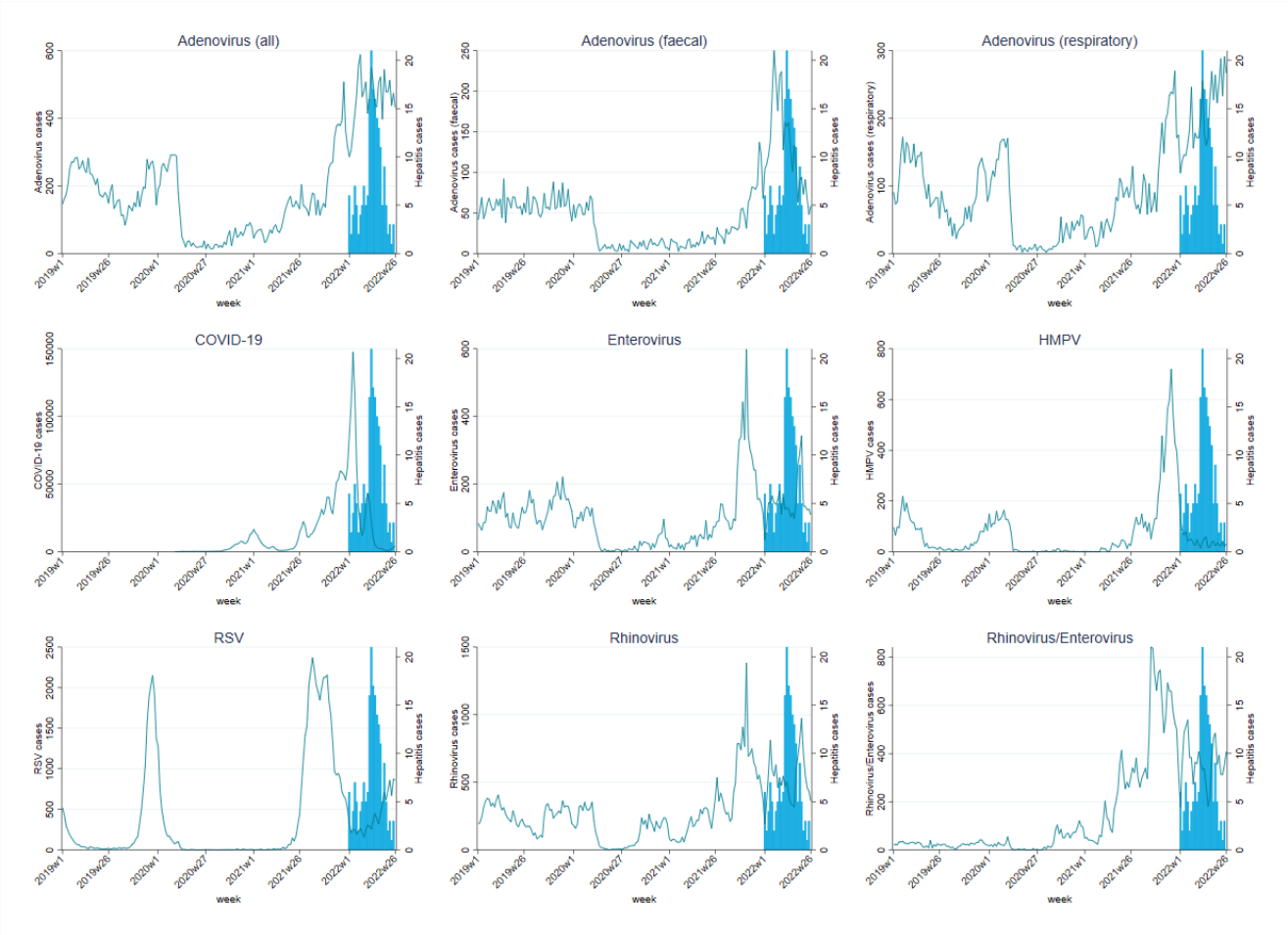
Figure S1: Serum SARS-CoV-2 nucleocapsid or spike protein antibody positivity in hepatitis cases and age-matched community comparison group, England



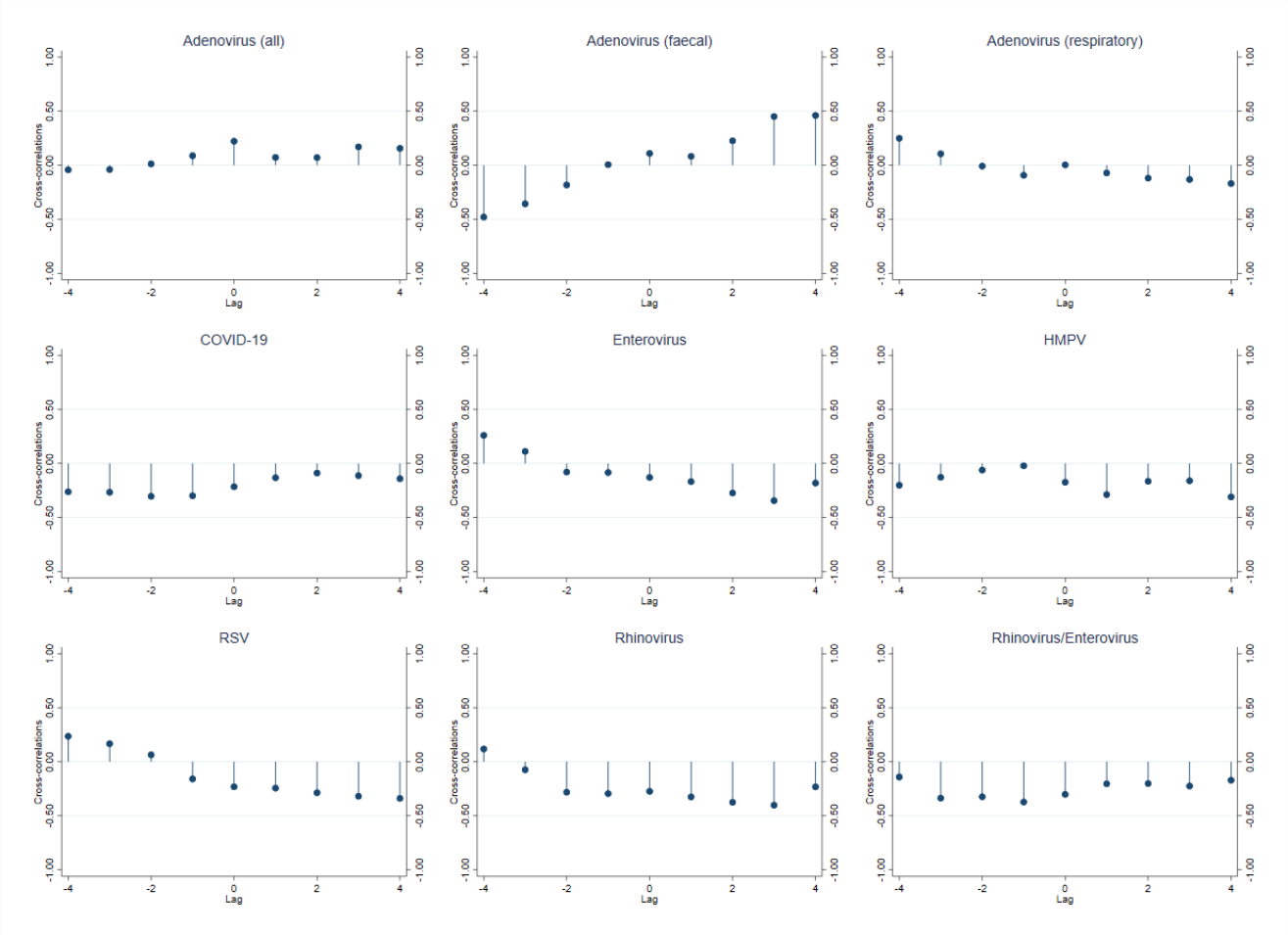
Comparison group from contemporaneous age-matched samples held at the UK Health Security Agency Sero-Epidemiological Unit (SEU)

Figure S2: Trends in detection of circulating viruses in national surveillance of laboratory reports and acute hepatitis cases, 2019 to week 26 2022, England



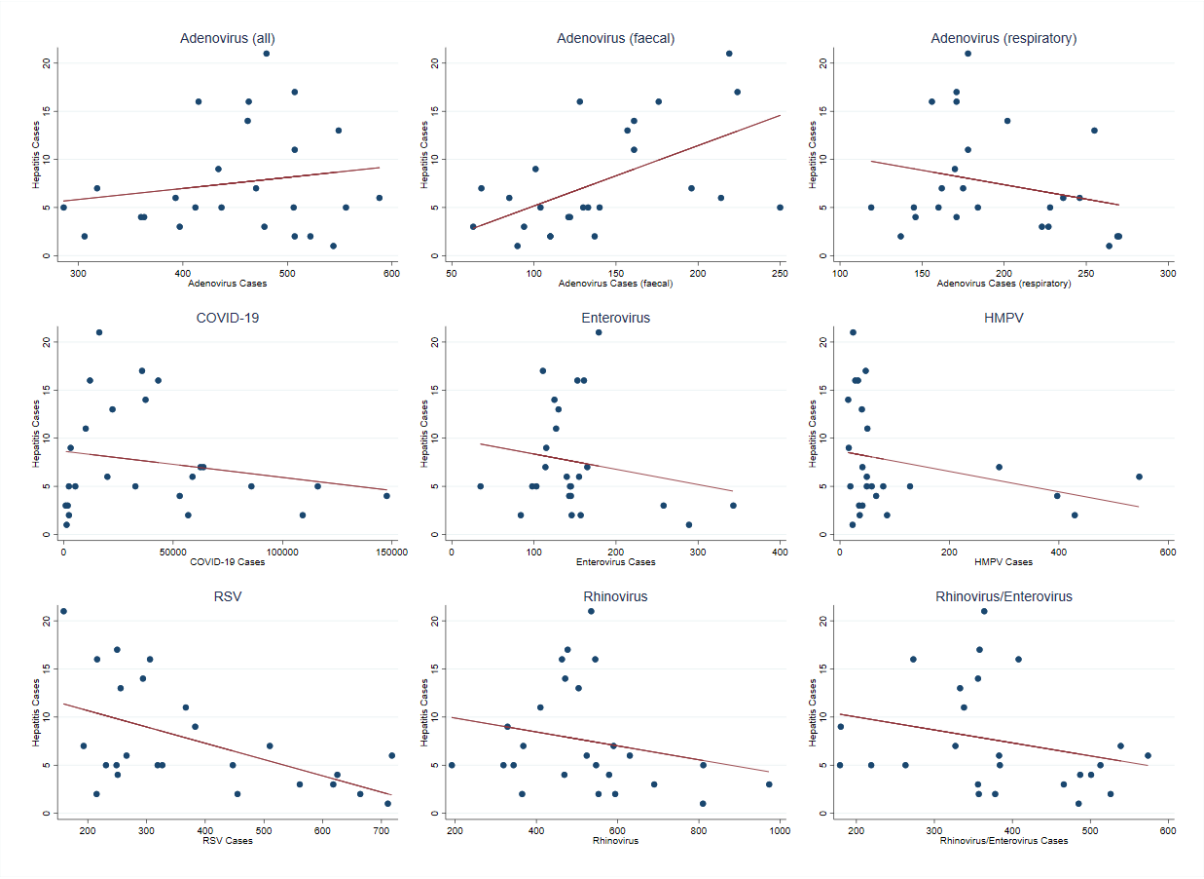
Diagnostic laboratory pathogen reporting to the Second Generation Surveillance System, UK Health Security Agency.

Figure S3: Cross correlogram of circulating viruses with hepatitis cases with up to +/- 4 weeks lag or lead, England 2022



Diagnostic laboratory pathogen reporting to the Second Generation Surveillance System, UK Health Security Agency.

Figure S4: Scatter plots of circulating pathogens in diagnostic laboratory surveillance and acute hepatitis cases at a four-week lag, England 2022



Includes line of best fit. Diagnostic laboratory pathogen reporting to the Second Generation Surveillance System, UK Health Security Agency.

Table S1: List of pathogens recommended for local investigations.

Sample type	Test	Pathogen
Blood	PCR	Adenovirus, Enterovirus, CMV, EBV, HSV, Hepatitis A, Hepatitis C, Hepatitis E, HHV6 and 7
Blood	Serology	Hepatitis A, B, C, E, CMV, EBV, SARS-CoV-2 anti-S, SARS-CoV-2 anti-N (only if locally available)
Blood	Culture	Standard culture for bacteria/fungi (only if clinically indicated i.e. fever)
Throat swab	PCR	Respiratory virus panel (including adenovirus/enterovirus/influenza, SARS-CoV-2)
Stool	PCR	Adenovirus, sapovirus, norovirus, enterovirus Standard bacterial stool pathogen panel to include Salmonella spp (or stool culture depending on local test availability)
Blood (whole blood in EDTA and plasma separated specimens)	Toxicology	Local investigations according to history Store samples locally - UKHSA will contact laboratories to request samples
Urine	Toxicology	Local investigations according to history Store samples locally - UKHSA will contact laboratories to request samples

Table S2: Characteristics of all cases by adenovirus infection status, all nations, United Kingdom (UK)

	Adenovirus-positive % n	Adenovirus-negative % n	p-value
Sex			0.28
Male	46.5% (79/170)	53.8% (43/80)	
Female	53.5% (91/170)	46.3% (37/80)	
Age (years)			<0.001
≤5	85.4% (146/171)	65.0% (52/80)	
6-10	14.6% (25/171)	22.5% (18/80)	
11-15	0	12.5% (10/80)	
Region or nation of residence			0.008
East Midlands	6.0% (10/168)	6.3% (5/80)	
East of England	6.5% (11/168)	11.3% (9/80)	
London	4.2% (7/168)	12.5% (10/80)	
North East	8.3% (14/168)	2.3% (2/80)	
North West	10.7% (18/168)	7.5% (6/80)	
South East	10.7% (18/168)	11.3% (9/80)	
South West	8.9% (15/168)	5.0% (4/80)	
West Midlands	7.1% (12/168)	7.5 (6/80)	
Yorkshire and the Humber	8.3% (14/168)	5.0% (4/80)	
Wales	10.7% (18/168)	0	
Northern Ireland	7.1% (12/168)	15.0% (12/80)	
Scotland	11.3% (19/168)	16.3% (13/80)	
Highest level of care			0.45
Transplant	7.6% (12/157)	2.7% (2/75)	
PICU	29.3% (46/157)	30.7% (23/75)	
Hospitalized	60.5% (95/157)	65.3% (49/75)	
Not hospitalized	2.5% (4/157)	1.3% (1/75)	
Peak Bilirubin*	125 (88-191)	76 (11-141)	<0.001
Peak AST ^*	2958 (1735-4226)	949 (467-2005)	<0.001
Peak ALT ^*	2496 (1686-3507)	1053 (714-2077)	<0.001
Abnormal Clotting (INR>2, PT>14s)	45.1% (51/113)	37.8% (17/45)	0.40

^ median; *England & Scotland only; INR = international normalized ratio; PT = prothrombin time

Table S3: Comparison of adenovirus positivity, January to May 2017 to 2019 and January to May 2022, by age group, Wales

	Year (January to May period)	Age group (years)		
		<5	5-9	10-24
Confirmed adenovirus cases detected (n)	2017-19	504	75	69
	2022	969	109	48
Total respiratory samples tested (n)	2017-19	4324	867	1761
	2022	5138	1156	2437
Adenovirus positivity (%)	2017-19	11.7%	8.7%	3.9%
	2022	18.9%	9.4%	2.0%
P value (difference in positivity between 2017-19 and 2022)		<0.0001	0.55	<0.0001

Table S4: Multivariable unconditional logistic regression for case-control analysis among those HHV-6 positive, England and Wales

		Cases n (%)	Controls n (%)	Multivariable OR	95%CI
Total		16	63	-	-
Sex					
	Male	8 (50%)	34 (54%)	1	
	Female	8 (50%)	29 (46%)	0.8	0.1-4.7
Age (years)					
	1 year	1 (6%)	14 (22%)	1	
	2 years	2 (13%)	13 (21%)	0.84	0.1-12.8
	3 years	6 (38%)	20 (32%)	8.38	0.7-94.3
	4 years	2 (13%)	6 (10%)	14.92	0.2-1310.7
	5 to 10 years	5 (31%)	10 (16%)	13.93	0.7-274.5
Date of presentation					
	January/February	1 (6%)	19 (31%)	1	
	March/April	13 (81%)	43 (69%)	3.9	0.3-51.9
	May/June	2 (13%)	0 (0%)	63.5	0.5-7667.5
Region					
	North England	4 (25%)	23 (37%)	1	
	Midlands (England)	3 (19%)	10 (16%)	1.41	0.1-19.9
	South England	6 (38%)	15 (24%)	4.02	0.4-40.7
	Wales	3 (19%)	15 (24%)	1.08	0.1-19.7
Adenovirus DNA					
	Detected	15 (94%)	11 (17%)	47.4	6.1-367.9
	Not detected	1 (6%)	52 (83%)	1	

DNA =Deoxyribonucleic acid

Table S5: Multivariable unconditional logistic regression for case-control analysis among those HHV-6 negative, England and Wales

		Cases n (%)	Controls n (%)	Multivariable OR	95%CI
Total		**16**	**81**	**-**	**-**
Sex					
	Male	9 (56%)	47 (58%)	1	
	Female	7 (44%)	34 (42%)	0.7	0.2-3.0
Age (years					
	1 year	1 (6%)	8 (10%)	1	
	2 years	3 (19%)	11 (14%)	4.2	0.2-97.3
	3 years	4 (25%)	12 (15%)	2.5	0.1-42.6
	4 years	4 (25%)	16 (20%)	3.1	0.2-61.2
	5 to 10 years	4 (25%)	34 (42%)	1.5	0.1-30.9
Date of presentation					
	January/February	6 (38%)	25 (31%)	1	
	March/April	9 (56%)	56 (69%)	0.4	0.1-1.6
	May/June	1 (6%)	0 (0)	1.2	0.02-84.9
Region					
	North England	7 (44%)	29 (36%)	1	
	Midlands (England)	1 (6%)	12 (15%)	0.3	0.02-4.4
	South England	3 (19%)	30 (37%)	0.3	0.04-1.6
	Wales	5 (31%)	10 (12%)	1.5	0.2-9.4
Adenovirus DNA					
	Detected	13 (81%)	11 (14%)	15.5	4.0-60.5
	Not detected	3 (19%)	70 (86%)	1	

DNA =Deoxyribonucleic acid

Table S6: Univariable unconditional logistic regression for case-control analysis, stratified by age to investigate impact of age alone on effect size, all UK nations

		Univariable		
		n	**OR**	**95%CI**
Total		**239**	**-**	**-**
Age (years)				
	1	32	9	0.6-494
	2	35	21.7	2.05-1012
	3	61	42	8.0-246
	4	47	50	6.8-530
	≥5	64	360	25.4-15366

Table S7: Multivariable unconditional logistic regression for case-control analysis, including ethnic group, England

		Cases n (%)	Controls n (%)	Multivariable OR	95%CI
Total		**57**	**119**	-	-
Sex					
	Male	28 (49%)	70 (59%)	1	
	Female	29 (51%)	49 (41%)	1.2	0.5-3.1
Age (years					
	1 year	3 (5%)	14 (12%)	1	
	2 years	8 (14%)	17 (14%)	1.9	0.3-10.7
	3 years	20 (35%)	29 (24%)	8.3	1.5-45.8
	4 years	13 (23%)	18 (15%)	5.5	0.9-31.4
	5 to 10 years	13 (23%)	41 (34%)	3.8	0.7-20.5
Date of presentation					
	January/February	15 (26%)	34 (29%)	1	
	March/April	42 (74%)	85 (71%)	0.5	0.2-1.3
	May/June	0	0		
Region					
	North England	19 (33%)	52 (44%)	1	
	Midlands (England)	12 (21%)	22 (18%)	1.1	0.3-3.9
	South England	26 (46%)	45 (38%)	1.4	0.5-3.9
Ethnic Group					
	White	53 (93%)	96 (83%)	1	
	Non-White	4 (7%)	20 (17%)	0.5	0.1-2.3
Adenovirus DNA					
	Detected	49 (86%)	18 (15%)	36.7	13.35-100.76
	Not detected	8 (14%)	101 (85%)	1	

DNA = Deoxyribonucleic acid

Table S8: Multivariable conditional logistic regression for case-control analysis - all UK nations

	Multivariable	
	OR	**95%CI**
Adenovirus DNA		
Detected	43.0	16.0-115.4
Not detected	1	

DNA =Deoxyribonucleic acid

Adenovirus variable and cases/controls as per Table 3.

Table S9: Multivariable unconditional logistic regression for case-control analysis - All blood specimens regardless of sample types, UK nations

		Cases n (%)	Controls n (%)	Multivariable OR	95%CI
Total		**103**	**246**	**-**	**-**
Sex					
	Male	49 (48%)	126 (51%)	1	
	Female	54 (52%)	120 (49%)	0.9	0.4-1.6
Age (years)					
	1 year	7 (7%)	49 (21%)	1	
	2 years	16 (16%)	42 (18%)	2.1	0.7-6.4
	3 years	31 (30%)	48 (20%)	5.8	2.0-16.9
	4 years	26 (25%)	33 (14%)	5.4	1.7-16.9
	5-10 years	23 (22%)	66 (28%)	3.8	1.3-11.2
Date of presentation					
	January/February	20 (19%)	58 (24%)	1	
	March/April	73 (71%)	174 (71%)	0.7	0.3-1.6
	May/June	10 (10%)	13 (5%)	2.2	0.6-8.8
Region					
	North England	19 (18%)	52 (21%)	1	
	Midlands (England)	12 (12%)	22 (9%)	1.1	0.3-3.4
	South England	26 (25%)	45 (18%)	1.4	0.6-3.7
	Wales	13 (13%)	45 (18%)	0.7	0.2-2.0
	Northern Ireland	9 (9%)	22 (10%)	1.2	0.3-4.1
	Scotland	24 (23%)	60 (24%)	3.4	1.2-9.6
Adenovirus DNA					
	Detected	78 (76%)	33 (13%)	25.9	12.8-52.5
	Not detected	25 (24%)	213 (87%)	1	

DNA =Deoxyribonucleic acid

S10: Ethics

In England, Regulation 3 of The Health Service (Control of Patient Information) Regulations 2002 gives the public health agency (UKHSA) legal permission to process patient confidential information for national surveillance of communicable diseases and, as such, individual patient consent is not required to access records on cases.

In Scotland, the Public Health etc. (Scotland) Act 2008 and the NHS Scotland Act 1978 gives the public health agency legal permission to process patient information for national surveillance of communicable diseases.

For England and Scotland ,approval was obtained from the UKHSA Research Ethics Governance Group that the case-control study was part of outbreak response.

Public Health Wales (PHW) was established under the "The Public Health Wales National Health Service Trust (Establishment) Order 2009". Its functions include the provision and management of a range of public health, health protection, healthcare improvement, health advisory, child protection and microbiological laboratory services and services relating to the surveillance, prevention, and control of communicable diseases. Approval was obtained via the PHW Research and Development Office that this study was part of outbreak response and no further NHS permissions were required.

In Northern Ireland a specific data sharing agreement was established between the Regional Virology Laboratory and Public Health Agency, and the UK Health Security Agency, with approval that the case-control study was part of the incident response.