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Increasing rhinovirus prevalence in paediatric intensive care patients since the SARS-CoV2 pandemic

Eliza Gil [a,b,c,*], Sunando Roy [c], Tim Best [b], James Hatcher [b], Judith Breuer [b,c]

[a] Department of Clinical Research, London School of Hygiene and Tropical Medicine
[b] Department of Microbiology, Virology & Infection Control, Great Ormond Street Hospital for Children, UK
[c] Infection, Immunity and Inflammation Department, GOS Institute of Child Health, University College London, London, UK

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ABSTRACT

Background: Rhinovirus (HRV) is a significant seasonal pathogen in children. The emergence of SARS-CoV2, and the social restrictions introduced in, disrupted viral epidemiology. Here we describe the experience of Great Ormond Street Hospital (GOSH), where HRV almost entirely disappeared from the paediatric intensive care units (PICU) during the first national lockdown and then rapidly re-emerged with a fast-increasing incidence, leading to concerns about possible nosocomial transmission in a vulnerable population.

Objectives: To describe alterations in HRV infection amongst PICU patients at GOSH since the emergence of SARS-COV2

Study Design: 10,950 nasopharyngeal aspirate viral PCR samples from GOSH PICU patients from 2019 to 2023 were included. 3083 returned a positive result for a respiratory virus, with 1530 samples positive for HRV. 66 HRV isolates from August 2020 – Jan 2021, the period of rapidly increasing HRV incidence, were sequenced. Electronic health record data was retrospectively collected for the same period.

Results: Following a reduction in the incidence of HRV infection during the first national lockdown, multiple genotypes of HRV emerged amongst GOSH PICU patients, with the incidence of HRV infection rapidly surging to levels higher than that seen prior to the emergence of SARS-CoV2 and continuing to circulate at increased incidence year-round.

Conclusions: The incidence of HRV infection amongst GOSH PICU patients is markedly higher than prior to the emergence of SARS-CoV2, a pattern not seen in other respiratory viruses. The increased burden of HRV-infection in vulnerable PICU patients has both clinical and infection prevention and control Implications.

1. Introduction

Rhinovirus, a member of the *Picornaviridae* family, is a non-enveloped virus with an icosahedral capsid and positive single-stranded RNA genome [1–3]. HRV is classified into three species: HRV-A, HRV-B and HVR-C [4,5] with over 160 known serotypes [6,7].

HRV are the predominant cause of the common cold and are also frequently detected in individuals reporting no symptoms [8,9]. However, this belies HRV's potential virulence: HRV is a major cause of upper and lower respiratory tract infections and is a particularly significant pathogen in young children, in whom it is associated with both hospitalisation and poor outcome from acute respiratory illness [10–12]. Notably, HRV bronchiolitis poses a particularly high risk of subsequent development of asthma [13–19] and HRV infection is also a frequent precipitant of acute asthma exacerbations [20,21]. In addition, HRV is commonly detected in febrile infants with no other detectable cause [22]. Infection with HRV poses a significant burden to healthcare resources [23,24]. No vaccine or specific antiviral treatment exists; HRV infection management is therefore limited to supportive care [18].

Disease severity may not be equally distributed across the HRV species. HRV-B is least prevalent and most frequently isolated from asymptomatic individuals; HRV- A and HRV-C are more prevalent, and infection is more commonly associated with clinical illness [7,25–27]. HRV-C is particularly associated with worse manifestations of infection [26]. HRV infections are seasonal, with a large peak in incidence in autumn, perhaps associated with the return of children to school, and a smaller peak in the spring [28–30]. However, there also appear to be distinctions in the seasonality of HRV species, with HRV-C

* Corresponding author at: Department of Clinical Research, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, UK.
E-mail address: eliza.gil@lshtm.ac.uk (E. Gil).

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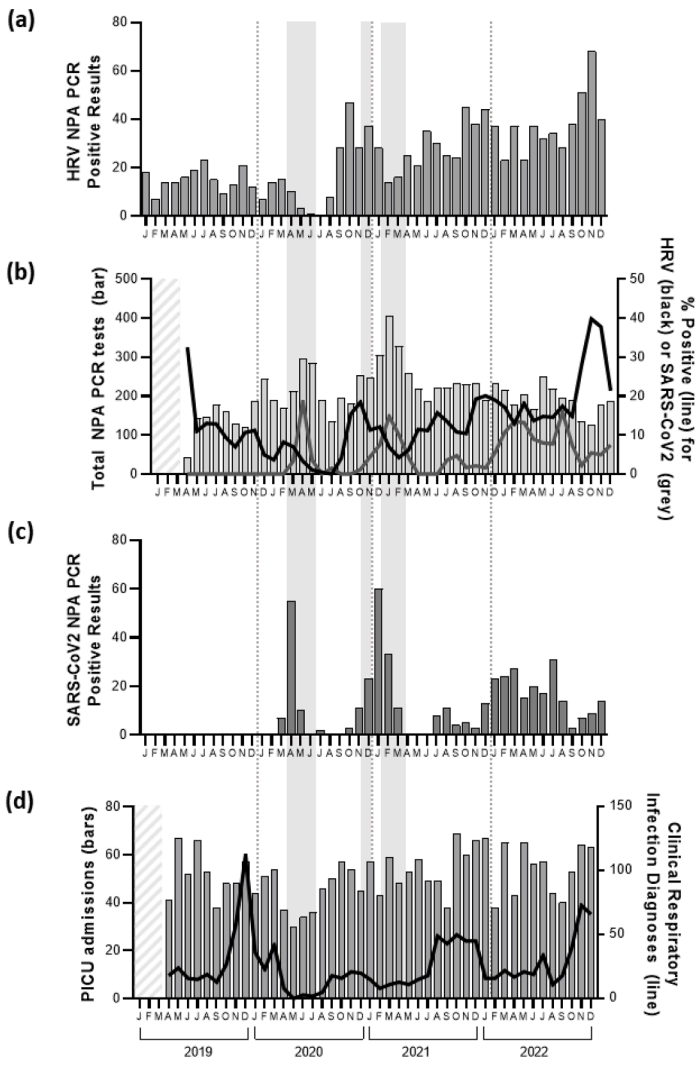

Fig. 1. (a) Number of NPA PCR results positive for HRV amongst GOSH PICU patients by month from 2019 to 2022. Years separated by dotted lines; grey shaded areas represent the time periods of national lockdown measures. (b) Total NPA PCR tests performed on SOH PICU patients (bars, plotted against left Y axis) and the percentage of these NPA PCR tests positive for HRV (black line, plotted against right Y axis) or SARS-CoV2 (grey line, plotted against right Y axis), the hashed area represents the time before an electronic health record was introduced and total test numbers could not be obtained. (c) Number of NPA PCR results positive for SARS-CoV2 amongst GOSH PICU patients (d) Number of patients admitted to GOSH PICU (bars, plotted against left Y axis) and number of clinical respiratory infection diagnoses (black line, plotted against right Y axis), hashed area represents the time before an electronic health record was introduced and admission and clinical data could not be obtained. (e) Number of clinical respiratory infection diagnoses at different prevalence of HRV infection detected by NPA PCR, linear regression performed by GraphPad Prism 9: solid line $Y = 0.7642*X + 15.90$, 95% CI dotted lines, slope significantly non-zero, r^2 0.097, p 0.0375. (f) Number of patients admitted to GOSH PICU at different prevalence of HRV infection detected by NPA PCR, linear regression performed by GraphPad Prism 9: solid line $Y = 0.4523*X + 45.38$, 95% CI dotted lines, slope significantly non-zero, r^2 0.097, p 0.0088.

predominantly detected in winter, while HRV-A is detected year round [19].

HRV is usually transmitted through aerosols or droplets or alternatively via contact with contaminated hands or objects [28,31–34]. The lockdown measures introduced in 2020 in response to the global SARS-CoV2 pandemic would be expected to reduce the transmission of other respiratory viruses, including HRV [35]. Consistent with this, a reduction in the incidence of many respiratory viruses including HRV was observed, however, HRV continued to be detected in several geographic locations throughout [36–47]. In countries where the HRV incidence had fallen during lockdown, it typically rose again to prior levels when restrictions were lifted [44–51]. Interestingly, in Australia HRV appeared to reach incidence levels higher than that seen before the SARS-CoV2 pandemic [51]. This study describes the rapid re-emergence, and subsequent increasing burden, of HRV infection amongst GOSH PICU patients since the lifting of the first national SARS-COV2 lockdown, a pattern that has not been seen in other respiratory viruses.

2. Methods

A retrospective search for all nasopharyngeal aspirate (NPA) viral PCR results from PICU patients January 2019 to December 2023 was performed on the GOSH laboratory information management system, yielding 10,950 results, 3083 of which were positive for a respiratory virus, 1530 of which were positive for HRV. These samples had been tested as per GOSH routine diagnostic testing protocols, using the Hamilton STAR platform for automated nucleic acid extraction and PCR set-up, followed by PCR with Takara mastermix on a Quantstudio 5 thermal cycler within-house primers and probes validated through External Quality Assurance. This was consistent throughout the study period. For all HRV positive samples, the full PCR panel result was obtained including cycle threshold (CT) values for HRV and all co-infecting viruses.

PICU admission numbers and clinical respiratory infection diagnoses were obtained through retrospective searching of the EPIC electronic health record (EHR) system from April 2019, when the EHR was introduced, until December 2022.

When time periods were used, they were defined as per below:

	Start	End	Duration (days)
Pre-lockdown 1	01/01/2020	22/03/2020	81
Lockdown 1	23/03/2020	23/06/2020	92
Post-lockdown 1	24/06/2020	30/10/2020	128
Lockdown 2	31/10/2020	02/12/2020	32
Post-lockdown 2	03/12/2020	05/01/2021	33
Lockdown 3	17/05/2021	18/07/2021	62
Post-Lockdown 3	19/07/2021	31/10/2021	104

The spatial location of the patient and timing of samples were used for initial clustering. Subsequently, 66 HRV samples were randomly selected from GOSH patients primarily from PICU, between 28/8/20

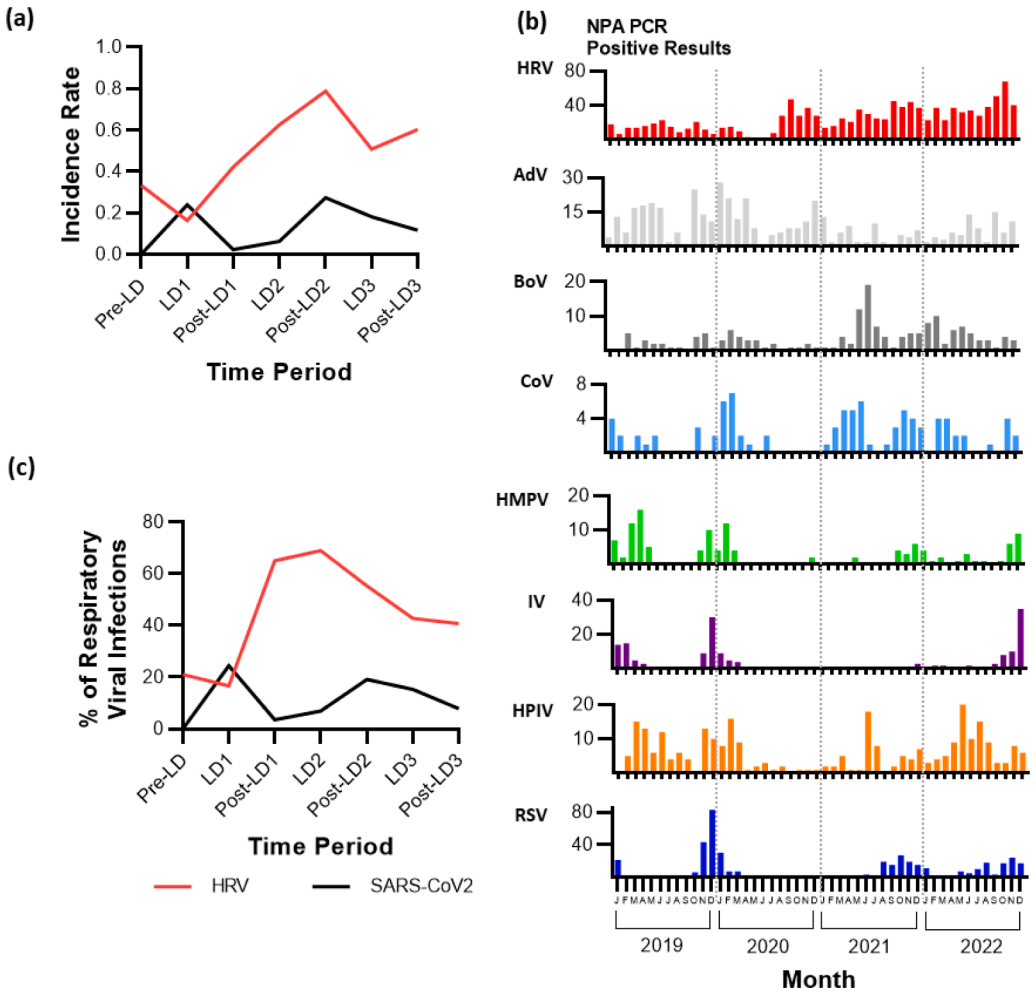

Fig. 2. (a) Incidence rate (new cases per day) of HRV (red line) and SARS-CoV2 (black line) in GOSH PICU patients during the time periods of the pandemic, as defined in the methods section: pre-lockdowns (Pre-LD), Lockdowns (LD) 1/2/3, and post lockdown periods (post-LD) (b) Number of NPA PCR positive results from GOSH PICU patients for HRV: rhinovirus, AdV: adenovirus, BoV: bocavirus, CoV: seasonal coronaviruses, HMPV: human metapneumovirus, IV: influenza, HPIV: parainfluenza and RSV: respiratory syncytial virus, by month from 2019 to 2022. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and 28/1/21, the period of rapidly increasing HRV incidence, for whole genome sequencing.

Viral whole genome sequencing was carried out by bait capture with biotinylated RNA oligonucleotides used in the Agilent SureSelectXT (SSXT) protocols. A design targeting DNA and RNA respiratory viruses (Coronavirus, Influenza A/B, Measles, Mumps, Rubella, RSV A/B, Rhinovirus, Metapneumovirus, Parainfluenza virus, Adenovirus and Bocavirus) was designed in-house and synthesised by Agilent Technologies (available through Agilent's Community Designs (NGS) programme as the SureSelectXT CD Pan Respiratory panel).

11 μl RNA extract was used in first-strand cDNA synthesis with random primers and SuperScript IV (Thermo Fisher Scientific) according to the manufacturer's instructions. Second-strand cDNA was synthesised using the NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module (New England BioLabs) according to the manufacturer's instructions. Double-stranded cDNA was purified using AMPure XP Beads (Beckman Coulter) with a 40 μl elution volume and was quantified using a high-sensitivity dsDNA Qubit kit (Fisher Scientific).

Double stranded cDNA (bulked with male human gDNA (Promega) if required) was sheared using a Covaris E220 focused ultra-sonication system (42 s, PIP 75, duty factor 10, cycles per burst 1000). End-repair, non-templated addition of 3′ poly A, adaptor ligation, pre-capture PCR, hybridisation, post-capture PCR and all post-reaction clean-up steps were performed using the SureSelectXT Low Input kit (Agilent Technologies) with minor modifications to the manufacturer's protocol to account for variable pathogen loads. Quality control steps were performed using the 4200 TapeStation (Agilent Technologies). Samples were sequenced using the MiSeq platform (Illumina). Base calling and sample demultiplexing were performed onboard the MiSeq generating paired FASTQ files for each sample.

The reads were first quality checked for low quality reads, and ambiguous bases and adapters trimmed from ends. The trimmed reads were mapped to a curated panel of Rhinovirus genotypes to identify the best reference genotype to use for mapping. Genotypes were assigned based on the genotype of the best reference match. The top reference genotype was used to remap the trimmed reads. Duplicate reads were then removed, and consensus sequences were called at 10X minimum threshold. The entire analysis was carried out using CLC Genomics Workbench 11.01. 26 (39.4%) complete genomes with high read depth were assembled and typed.

As the polymerase of HRV is highly error prone, we considered 30 bp to be adequate to determine relatedness over a 3 week period, based on accumulation of ~30 mutations at an approximate rate of 1.5×10^{-4} mutation per nucleotide per replication cycle over the genome and taking a replication cycle of 8 hrs. Therefore, 3 criteria were used to identify possible linked cases:

i Genetic similarity: pairwise polymorphic difference <30 bp
ii Time: sampling date within 3 weeks of each other
iii Place: sampling done in the same ward

3. Results

The first COVID lockdown in the UK, which began on the 23rd March 2020, led to a marked decline in the detection of HRV by NPA PCR in PICU patients at GOSH (Fig. 1a). Following the end of national lockdown

Table 1
Rhinovirus genotype results of the 26 samples successfully sequenced.

Sample	Genotype
1	C15
2	A49
3	C43
4	B83
5	C45
6	C15
7	A47
8	C
9	C42
10	C15
11	A22
12	C11/New Recombination
13	C11/New Recombination
14	C
15	B42
16	C1
17	B1
18	A47
19	A49
20	C43
21	A11
22	A61
23	A49
24	A49
25	A
26	A

Table 2
Samples of the same genotype, which of the criteria they met to be considered as possibly linked: i. Genetic similarity: pairwise polymorphic difference <30 bp, ii. Time: sampling date within 3 weeks of each other, iii. Place: sampling done in the same ward; and which of the samples fulfilled this criterion.

Genotype	Samples	Criteria fulfilled: relevant sample numbers
A49	2, 19, 23,24	i: 2, 19
C15	1, 6, 10	i, ii & iii: 1, 6
C43	3, 20	i: 3, 20
A47	7, 18	i: 7, 18
C11	12, 13	
A	25, 26	
C	8, 14	i: 8, 14

measures on the 23rd June 2020 there was a rapid increase in both incidence (Fig. 1a) and prevalence (Fig. 1b) of HRV. This was not the result of increased testing (Fig. 1b). SARS-CoV2 took longer than HRV to re-emerge following the lifting of the first national lockdown measures (Fig. 1c). This rise in HRV detection occurred during a period of increasing PICU admissions, as well as an increase, albeit small, in clinical diagnoses of respiratory infection (Fig. 1d). Over the study period, the prevalence of HRV as detected by NPA PCR correlated with the number of clinical diagnoses of respiratory infection amongst PICU patients at GOSH (Fig. 1e), as well as with the number of PICU admissions (Fig. 1f).

Considering the time periods of the pandemic incidence rate of HRV infection amongst GOSH PICU patients dropped during the first and third lockdowns, but not the shorter second lockdown and rose each time following the lifting of lockdown measures (Fig. 2a) with a marked and prolonged increasing incidence rate (Fig. 2b). This pattern was not mirrored by SARS-CoV2 (Fig. 2a). The pattern of persistently increasing cases following the lifting of national restrictions was not seen in other respiratory viruses (Fig. 2b), resulting in HRV accounting for a markedly higher proportion of respiratory viral infections (Fig. 2c).

Given the rapidity of the rise in cases of HRV within the PICU in the summer of 2020 (Fig. 1a), there was concern that this may represent a nosocomial HRV outbreak. There were no evident clusters of cases, either temporally or spatially to substantiate this hypothesis so

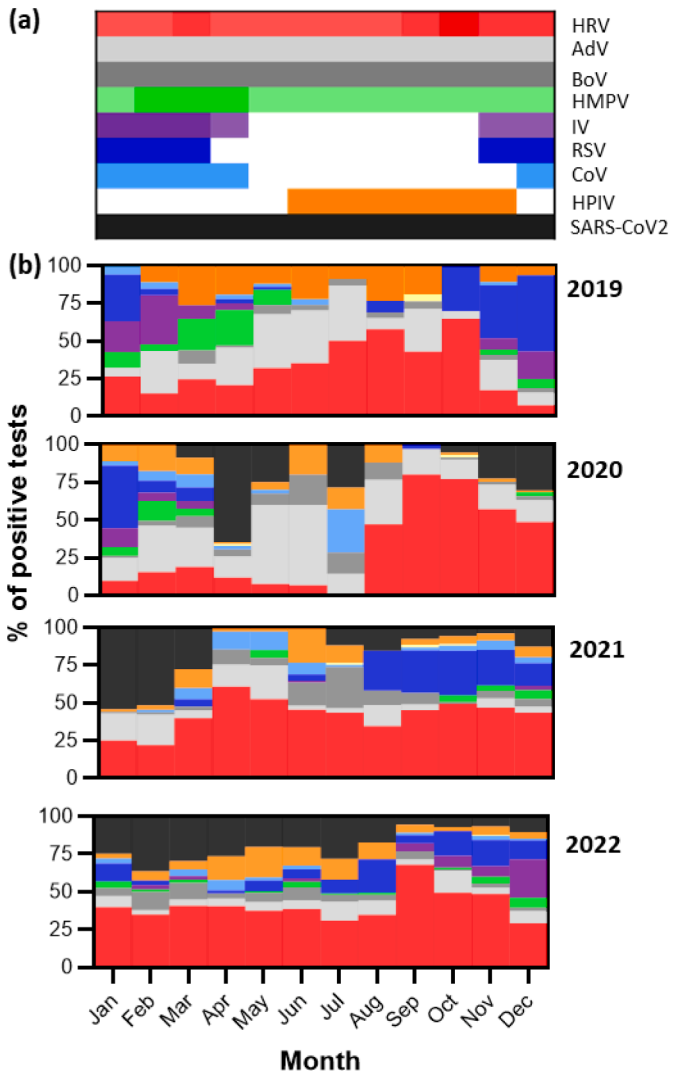

Fig. 3. (a) Typical seasonal pattern of respiratory viruses, based on [62]. (j) Proportion of positive nasopharyngeal PCR results attributable to each respiratory virus amongst GOSH PICU patients by month from 2019 to 2022.

genotypic analysis was performed. Of the 26 complete genomes assembled, the majority were HRV-C (12 samples) or HRV-A (11 samples), the remaining 3 were HRV-B (Table 1). Within each HRV species, there was a broad range of genotypes identified (Table 1).

Given the lack of evident phylogenetic linkage, we next looked for evidence of transmission pairs, using the criteria defined in Methods. Only 1 pair of samples, numbers 1 & 6, fulfilled all three criteria (Table 2), with a further four pairs meeting criteria i. genetic similarity (Table 2), however these sample pairs were separated in both time and place and could not be epidemiologically linked. The rapid increase in HRV cases amongst PICU cases therefore could not be attributed to an outbreak or a common source of infection.

Many respiratory viruses, including HRV, have a typical seasonality (Fig. 3a), which was reflected in the prevalence of respiratory viruses amongst GOSH PICU patients prior to the pandemic (Fig. 3b). Following the pandemic, HRV was detected year-round in 2021 and 2022, and no September peak in 2021 despite the normal opening of schools (Fig. 3b). The seasonality of several other respiratory viruses also deviated from the classical pattern, with notable alterations in RSV and IV seasonality, as well as markedly reduced detection of AdV (Figs. 2b & 3b).

HRV was frequently identified alongside another respiratory viral pathogen, here termed co-infection: this may represent true simultaneous active infection or detection of residual viral DNA/RNA due to a

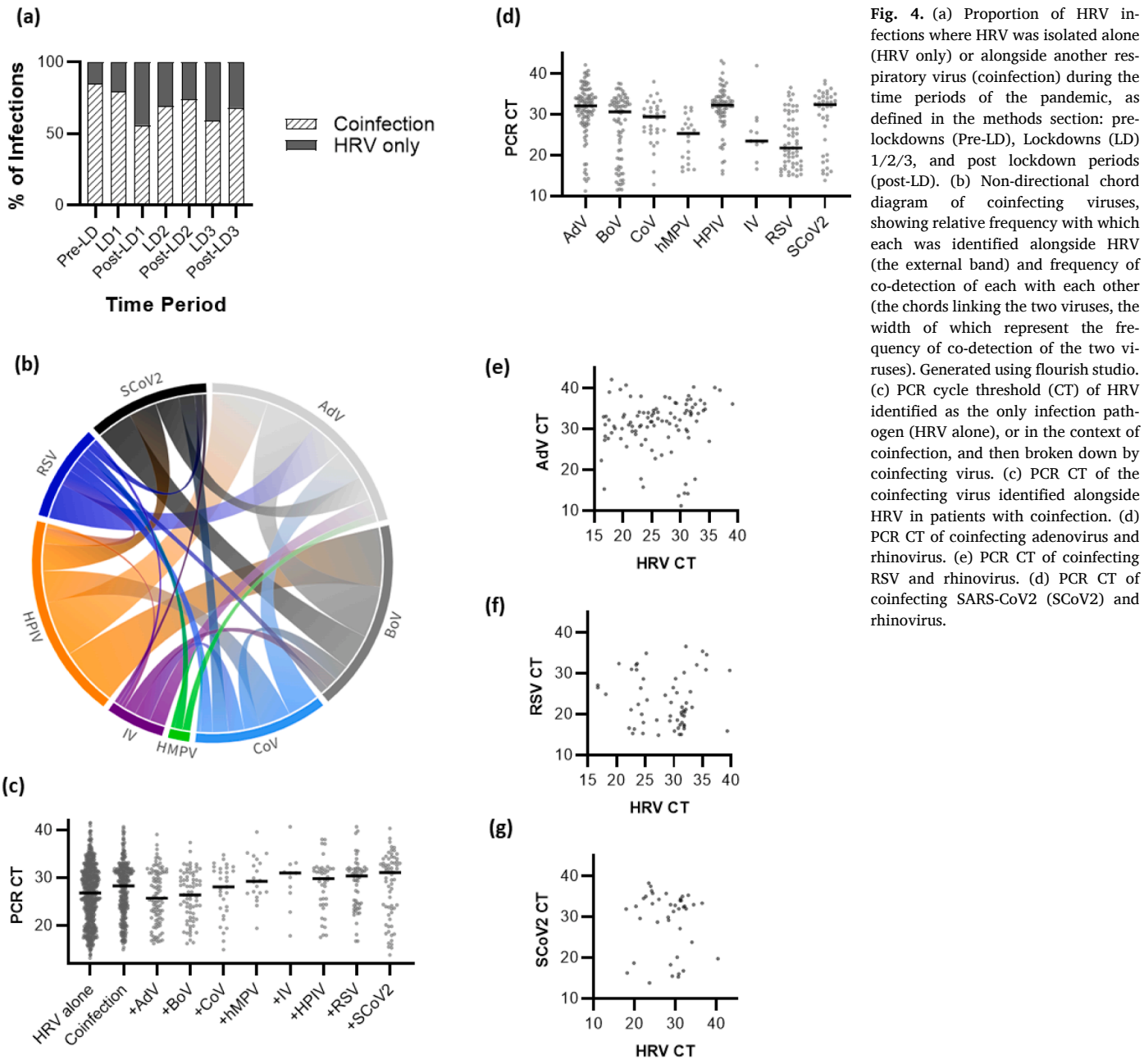

Fig. 4. (a) Proportion of HRV infections where HRV was isolated alone (HRV only) or alongside another respiratory virus (coinfection) during the time periods of the pandemic, as defined in the methods section: pre-lockdowns (Pre-LD), Lockdowns (LD) 1/2/3, and post lockdown periods (post-LD). (b) Non-directional chord diagram of coinfecting viruses, showing relative frequency with which each was identified alongside HRV (the external band) and frequency of co-detection of each with each other (the chords linking the two viruses, the width of which represent the frequency of co-detection of the two viruses). Generated using flourish studio. (c) PCR cycle threshold (CT) of HRV identified as the only infection pathogen (HRV alone), or in the context of coinfection, and then broken down by coinfecting virus. (c) PCR CT of the coinfecting virus identified alongside HRV in patients with coinfection. (d) PCR CT of coinfecting adenovirus and rhinovirus. (e) PCR CT of coinfecting RSV and rhinovirus. (d) PCR CT of coinfecting SARS-CoV2 (SCoV2) and rhinovirus.

Table 3
Coinfecting respiratory virus detected by PCR in the 312 patients simultaneously infected with more than one respiratory virus.

Coinfecting virus	Number patients	% of all HRV cases
Adenovirus	90	11.73
Bocavirus	71	9.26
Parainfluenza	67	8.74
RSV	50	6.52
SARS-CoV2	38	4.95
Coronavirus	30	3.91
Human metapneumovirus	17	2.22
Influenza	10	1.30

recent past infection with one or more of the pathogens identified. The relative frequency of co-infections fell as the incidence of HRV rose (Fig. 4a). Of the patients with HRV infection in the context of coinfection with at least on other respiratory viral pathogen, and no concern regarding the possibility of specimen contamination, 198 patients had 2 respiratory viruses detected (including HRV), 86 had 3 respiratory viruses detected, 22 had 4 respiratory viruses detected, 6 had 5 respiratory viruses detected and 1 patient had 6 respiratory viruses detected. Adenovirus was the most common coinfecting virus, with BoV and HPIV also frequently detected; conversely influenza was rarely identified in patients with HRV (Table 3). Given the context of the COVID pandemic, SARS-CoV2 was rarely identified alongside HRV, being detected in only 4.95% of cases. Co-infections occurred in myriad combinations (Fig. 4b).

There were notable differences in distributions of PCR cycle threshold (CT) values, a semi-quantitative measure of viral load [52], of HRV in the context of coinfection (Fig. 4b). The HRV CT was higher in coinfection compared to infection with HRV alone and there were also marked differences in HRV CT depending on the coinfecting respiratory virus (Fig. 4c). There were also differences in the PCR CT values of the coinfecting viruses (Fig. 4d), with notable differences between the relatively low burden of AdV (median CT 32.1, IQR 28.8–34.6) across a

range of HRV burdens (Fig. 4e), versus the higher burden of RSV (median 21.8, IQR 18.0–28.7), mostly at higher CT values of HRV (Fig. 4f). SARS-COV2 was mostly detected only at high CT in the context of a high HRV CT value (Fig. 4g).

4. Discussion

The first UK national lockdown markedly reduced the detection of HRV amongst GOSH PICU patients. Shortly after these measures were lifted, genetically diverse strains of HRV were identified amongst intensive care patients at GOSH, causing a rapidly increasing burden of infection with no evidence of an outbreak, suggesting HRV circulation was sufficient in the wider community throughout the period of social restrictions to preserve a broad diversity of HRV strains. Since this rapid reemergence, HRV has been identified in GOSH PICU patients at levels higher than prior to the national lockdown measures, with a loss of seasonality. This pattern appears to be unique to HRV and does not appear to be the result of increased testing. It is not clear what underlies this phenomenon. The clinical significance of the increased detection of HRV remains uncertain: the virus may be directly implicated in admission to PICU, a co-factor in the admission, or an incidental finding, for example in the case of recent past, early acute or asymptomatic infection.

The rapid reemergence of multiple highly diverse genotypes across all three HRV species amongst GOSH PICU patients upon the lifting of social restriction is consistent with the continued circulation of HRV throughout the first national lockdown. It has been postulated that facemasks could be less effective at filtering HRV out of exhaled air due to its relative small size [53], which may have facilitated ongoing transmission during the periods or lockdown and social distancing regulations. In addition, HRV is a non-enveloped virus and is therefore more resistant to alcohol based disinfectant solutions than enveloped viruses [54]. As such it may have been less successfully eradicated from hands by the alcohol hand hygiene approaches recommended during the SARS-CoV2 pandemic. Consistent with the hypothesized limitations of the efficacy of public health measures introduced against SARS-CoV2 in controlling HRV, there is evidence the HRV continued to circulate in several countries in spite of social restrictions that markedly suppressed SARS-CoV2 transmission [39–43,46,47]. However, it is notable that HRV detection within GOSH PICU patients fell markedly during the first national lockdown, and again, although to a lesser extent, during the third lockdown. This suggests that it is not simply that the social restrictions introduced in response to the SARS-CoV2 pandemic failed to control HRV transmission, but that only the most stringent, and prolonged, measures reduced the circulation of HRV and hence infection of GOSH PICU patients with HRV, and even during these periods a broad range of HRV continued to circulate in the wider community.

In addition, HRV has not simply rebounded to its prior level amongst GOSH PICU patients; instead HRV is being detected with an ever-increasing incidence, markedly higher than previously. The SARS-CoV2 pandemic, and the lockdown measures enforced in response, have been described to have disrupted the seasonality of several respiratory viruses [55–57], however, this pattern of persistently increased incidence has not been seen in any other respiratory virus in our cohort. It is possible that this reduced circulation of the viruses with which HRV has negative interactions, notably RSV [58–60], has facilitated its circulation at such high levels. Interestingly, in spite of the pandemic context, SARS-CoV2 was rarely identified alongside HRV, and typically only at high CT when it was present, consistent with proposed negative interactions between these viruses [61]. It is also notable that AdV is being detected at a much lower incidence than previously, despite being the commonest coinfecting virus alongside HRV in this study.

In conclusion, amongst GOSH PICU patients, following a period of suppression during the first national lockdown, multiple HRV genotypes rapidly emerged and have continued to circulate amongst these patients at higher levels than before the SARS-CoV2 pandemic, with increasing incidence and loss of seasonality. The marked increase in rates of HRV infection has both clinical and infection prevention and control implications, particularly in vulnerable paediatric populations, and, if this is occurring in a wider setting, the increased circulation of HRV is likely to pose a significant burden to healthcare resources.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] M.G. Rossmann, E. Arnold, J.W. Erickson, E.A. Frankenberger, J.P. Griffith, H. J. Hecht, et al., Structure of a human common cold virus and functional relationship to other picornaviruses, Nature 317 (6033) (1985) 145–153.
[2] G. Stanway, P.J. Hughes, R.C. Mountford, P.D. Minor, J.W. Almond, The complete nucleotide sequence of a common cold virus: human rhinovirus 14, Nucleic Acids Res. 12 (20) (1984) 7859–7875.
[3] A.T. Hadfield, W.m. Lee, R. Zhao, M.A. Oliveira, I. Minor, R.R. Rueckert, et al., The refined structure of human rhinovirus 16 at 2.15 A resolution: implications for the viral life cycle, Struct. Lond. Engl. 1993 5 (3) (1997) 427–441.
[4] A.C. Palmenberg, D. Spiro, R. Kuzmickas, S. Wang, A. Djikeng, J.A. Rathe, et al., Sequencing and analyses of all known human rhinovirus genomes reveal structure and evolution, Science 324 (5923) (2009) 55–59.
[5] V.P. Waman, P.S. Kolekar, M.M. Kale, U. Kulkarni-Kale, Population structure and evolution of rhinoviruses, PLoS ONE 9 (2) (2014) e88981.
[6] D. Blaas, R. Fuchs, Mechanism of human rhinovirus infections, Mol. Cell Pediatr. 3 (2016) 21.
[7] C. Esneau, A.C. Duff, N.W. Bartlett, Understanding rhinovirus circulation and impact on illness, Viruses 14 (1) (2022) 141.
[8] N. Principi, A. Zampiero, M. Gambino, A. Scala, L. Senatore, M. Lelii, et al., Prospective evaluation of rhinovirus infection in healthy young children, J. Clin. Virol. Off. Publ. Pan. Am. Soc. Clin. Virol. 66 (2015) 83–89.
[9] A. Ruohola, M. Waris, T. Allander, T. Ziegler, T. Heikkinen, O. Ruuskanen, Viral etiology of common cold in children, Finland, Emerg. Infect. Dis. 15 (2) (2009) 344–346.
[10] L. van der Linden, A.H.L. Bruning, X.V. Thomas, R.P. Minnaar, S.P.H. Rebers, J. Schinkel, et al., A molecular epidemiological perspective of rhinovirus types circulating in Amsterdam from 2007 to 2012, Clin. Microbiol. Infect. 22 (12) (2016) 1002.e9–1002.e14.
[11] S.B. Drysdale, A. Mejias, O. Ramilo, Rhinovirus - not just the common cold, J. Infect. 74 (1) (2017) S41–S46. Suppl.
[12] M.K. Iwane, M.M. Prill, X. Lu, E.K. Miller, K.M. Edwards, C.B. Hall, et al., Human rhinovirus species associated with hospitalizations for acute respiratory illness in young US children, J. Infect. Dis. 204 (11) (2011) 1702–1710.
[13] M.M.H. Kusel, N.H. de Klerk, P.G. Holt, T. Kebadze, S.L. Johnston, P.D. Sly, Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: a birth cohort study, Pediatr. Infect. Dis. J. 25 (8) (2006) 680.
[14] Z. Piotrowska, M. Vázquez, E.D. Shapiro, C. Weibel, D. Ferguson, M.L. Landry, et al., Rhinoviruses are a major cause of wheezing and hospitalization in children less than 2 years of age, Pediatr. Infect. Dis. J. 28 (1) (2009) 25–29.
[15] L. Müller, I. Mack, C. Tapparel, L. Kaiser, M.P. Alves, E. Kieninger, et al., Human rhinovirus types and association with respiratory symptoms during the first year of life, Pediatr. Infect. Dis. J. 34 (8) (2015) 907.
[16] H.C. Meissner, Viral bronchiolitis in children, N. Engl. J. Med. 374 (1) (2016) 62–72.
[17] T. Juvén, J. Mertsola, M. Waris, M. Leinonen, O. Meurman, M. Roivainen, et al., Etiology of community-acquired pneumonia in 254 hospitalized children, Pediatr. Infect. Dis. J. 19 (4) (2000) 293–298.
[18] E. Bizot, A. Bousquet, M. Charpié, F. Coquelin, S. Lefevre, J. Le Lorier, et al., Rhinovirus: a narrative review on its genetic characteristics, pediatric clinical presentations, and pathogenesis, Front. Pediatr. 9 (2021), 643219.
[19] S. Morikawa, U. Kohdera, T. Hosaka, K. Ishii, S. Akagawa, S. Hiroi, et al., Seasonal variations of respiratory viruses and etiology of human rhinovirus infection in children, J. Clin. Virol. 73 (2015) 14–19.
[20] D.J. Jackson, J.E. Gern, Rhinovirus infections and their roles in asthma: etiology and exacerbations, J. Allergy Clin. Immunol. Pract. 10 (3) (2022) 673–681.

[21] N. Khetsuriani, X. Lu, W.G. Teague, N. Kazerouni, L.J. Anderson, D.D. Erdman, Novel human rhinoviruses and exacerbation of asthma in children, Emerg. Infect. Dis. 14 (11) (2008) 1793–1796.
[22] A.J. Blaschke, E.K. Korgenski, J. Wilkes, A.P. Presson, E.A. Thorell, A.T. Pavia, et al., Rhinovirus in febrile infants and risk of bacterial infection, Pediatrics 141 (2) (2018), e20172384.
[23] S.A. Asner, A. Petrich, J.S. Hamid, D. Mertz, S.E. Richardson, M. Smieja, Clinical severity of rhinovirus/enterovirus compared to other respiratory viruses in children, Influenza Other Respir. Viruses 8 (4) (2014) 436–442.
[24] J. Fine, A. Bray-Aschenbrenner, H. Williams, P. Buchanan, J. Werner, The resource burden of infections with rhinovirus/enterovirus, influenza, and respiratory syncytial virus in children, Clin. Pediatr. (Phila) 58 (2) (2019) 177–184.
[25] J.E. Linder, D.C. Kraft, Y. Mohamed, Z. Lu, L. Heil, S. Tollefson, et al., Human rhinovirus C: age, season, and lower respiratory illness over the past 3 decades, J. Allergy Clin. Immunol. 131 (1) (2013) 69–77.
[26] D.W. Cox, J. Bizzintino, G. Ferrari, S.K. Khoo, G. Zhang, S. Whelan, et al., Human rhinovirus species C infection in young children with acute wheeze is associated with increased acute respiratory hospital admissions, Am. J. Respir. Crit. Care Med. 188 (11) (2013) 1358–1364.
[27] E.K. Martin, J. Kuypers, H.Y. Chu, K. Lacombe, X. Qin, B. Strelitz, et al., Molecular epidemiology of human rhinovirus infections in the pediatric emergency department, J. Clin. Virol. Off. Publ. Pan. Am. Soc. Clin. Virol. 62 (2015) 25–31.
[28] J.M. Gwaltney, J.O. Hendley, G. Simon, W.S. Jordan, Rhinovirus Infections in an Industrial Population, N. Engl. J. Med. 275 (23) (1966) 1261–1268.
[29] N.W. Johnston, S.L. Johnston, J.M. Duncan, J.M. Greene, T. Kebadze, P.K. Keith, et al., The September epidemic of asthma exacerbations in children: a search for etiology, J. Allergy Clin. Immunol. 115 (1) (2005) 132–138.
[30] R.H.M. Price, C. Graham, S. Ramalingam, Association between viral seasonality and meteorological factors, Sci. Rep. 9 (1) (2019) 929.
[31] E.C. Dick, L.C. Jennings, K.A. Mink, C.D. Wartgow, S.L. Inhorn, Aerosol transmission of rhinovirus colds, J. Infect. Dis. 156 (3) (1987) 442–448.
[32] C.C. Wang, K.A. Prather, J. Sznitman, J.L. Jimenez, S.S. Lakdawala, Z. Tufekci, et al., Airborne transmission of respiratory viruses, Science 373 (6558) (2021) eabd9149.
[33] L. Liu, Y. Li, P.V. Nielsen, J. Wei, R.L. Jensen, Short-range airborne transmission of expiratory droplets between two people, Indoor Air 27 (2) (2017) 452–462.
[34] B. Winther, K. McCue, K. Ashe, J. Rubino, J.O. Hendley, Rhinovirus contamination of surfaces in homes of adults with natural colds: transfer of virus to fingertips during normal daily activities, J. Med. Virol. 83 (5) (2011) 906–909.
[35] J.E. Kreger, M.B. Hershenson, Effects of COVID-19 and social distancing on rhinovirus infections and asthma exacerbations, Viruses 14 (11) (2022) 2340.
[36] A.C. Vittucci, L. Piccioni, L. Coltella, C. Ciarlitto, L. Antilici, E. Bozzola, et al., The disappearance of respiratory viruses in children during the COVID-19 pandemic, Int. J. Environ. Res. Public Health 18 (18) (2021) 9550.
[37] S. Sayed, A.R. Diwadkar, J.W. Dudley, J. O'Brien, D. Dvorin, C.C. Kenyon, et al., COVID-19 pandemic-related reductions in pediatric asthma exacerbations corresponded with an overall decrease in respiratory viral infections, J. Allergy Clin. Immunol. Pract. 10 (1) (2022) 91–99.
[38] E. Partridge, E. McCleery, R. Cheema, N. Nakra, S. Lakshminrusimha, D. J. Tancredi, et al., Evaluation of seasonal respiratory virus activity before and after the statewide COVID-19 shelter-in-place order in Northern California, JAMA Netw. Open 4 (1) (2021), e2035281.
[39] D. Wu, J. Lu, Z. Sun, L. Cao, Q. Zeng, Q. Liu, et al., Rhinovirus remains prevalent in school teenagers during fight against COVID-19 pandemic, Immun. Inflamm. Dis. 9 (1) (2021) 76–79.
[40] E. Takashita, C. Kawakami, T. Momoki, M. Saikusa, K. Shimizu, H. Ozawa, et al., Increased risk of rhinovirus infection in children during the coronavirus disease-19 pandemic, Influenza Other Respir. Viruses 15 (4) (2021) 488–494.
[41] D. Marriott, R. Beresford, F. Mirdad, D. Stark, A. Glanville, S. Chapman, et al., Concomitant marked decline in prevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and other respiratory viruses among symptomatic patients following public health interventions in Australia: data from St Vincent's hospital and associated screening clinics, Sydney, NSW, Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 72 (10) (2021) e649–e651.
[42] L. Rodgers, M. Sheppard, A. Smith, S. Dietz, P. Jayanthi, Y. Yuan, et al., Changes in Seasonal Respiratory Illnesses in the United States During the Coronavirus Disease 2019 (COVID-19) Pandemic, Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 73 (1) (2021) S110–S117. Suppl.
[43] A. Amaddeo, C. Cason, G. Cozzi, L. Ronfani, M. Comar, Social distancing measures for COVID-19 are changing winter season, Arch. Dis. Child. 106 (12) (2021) e47.
[44] S. Kitanovski, G. Horemheb-Rubio, O. Adams, B. Gärtner, T. Lengauer, D. Hoffmann, et al., Rhinovirus prevalence as indicator for efficacy of measures against SARS-CoV-2, BMC Public Health 21 (1) (2021) 1178.
[45] S. Poole, N.J. Brendish, A.R. Tanner, T.W. Clark, Physical distancing in schools for SARS-CoV-2 and the resurgence of rhinovirus, Lancet Respir. Med. 8 (12) (2020) e92–e93.
[46] S. Park, I.C. Michelow, Y.J. Choe, Shifting patterns of respiratory virus activity following social distancing measures for coronavirus disease 2019 in South Korea, J. Infect. Dis. 224 (11) (2021) 1900–1906.
[47] I. Kuitunen, M. Artama, M. Haapanen, M. Renko, Rhinovirus spread in children during the COVID-19 pandemic despite social restrictions-A nationwide register study in Finland, J. Med. Virol. 93 (10) (2021) 6063–6067.
[48] T. Launay, C. Souty, A.M. Vilcu, C. Turbelin, T. Blanchon, C. Guerrisi, et al., Common communicable diseases in the general population in France during the COVID-19 pandemic, PLoS ONE 16 (10) (2021), e0258391.
[49] M. Redlberger-Fritz, M. Kundi, S.W. Aberle, E. Puchhammer-Stöckl, Significant impact of nationwide SARS-CoV-2 lockdown measures on the circulation of other respiratory virus infections in Austria, J. Clin. Virol. Off. Publ. Pan. Am. Soc. Clin. Virol. 137 (2021), 104795.
[50] Q.S. Huang, T. Wood, L. Jelley, T. Jennings, S. Jefferies, K. Daniells, et al., Impact of the COVID-19 nonpharmaceutical interventions on influenza and other respiratory viral infections in New Zealand, Nat. Commun. 12 (1) (2021) 1001.
[51] S.G. Sullivan, S. Carlson, A.C. Cheng, M.B. Chilver, D.E. Dwyer, M. Irwin, et al., Where has all the influenza gone? The impact of COVID-19 on the circulation of influenza and other respiratory viruses, Australia, March to September 2020, Euro Surveill. Bull. Eur. Sur. Mal. Transm. Eur. Commun. Dis. Bull. 25 (47) (2020), 2001847.
[52] D.G. Ginzinger, Gene quantification using real-time quantitative PCR: an emerging technology hits the mainstream, Exp. Hematol. 30 (6) (2002) 503–512.
[53] N.H.L. Leung, D.K.W. Chu, E.Y.C. Shiu, K.H. Chan, J.J. McDevitt, B.J.P. Hau, et al., Respiratory virus shedding in exhaled breath and efficacy of face masks, Nat. Med. 26 (5) (2020) 676–680.
[54] C. Savolainen-Kopra, T. Korpela, M.L. Simonen-Tikka, A. Amiryousefi, T. Ziegler, M. Roivainen, et al., Single treatment with ethanol hand rub is ineffective against human rhinovirus–hand washing with soap and water removes the virus efficiently, J. Med. Virol. 84 (3) (2012) 543–547.
[55] S. Hamid, A. Winn, R. Parikh, J.M. Jones, M. McMorrow, M.M. Prill, et al., Seasonality of respiratory syncytial virus - United States, 2017-2023, MMWR Morb. Mortal Wkly Rep. 72 (14) (2023) 355–361.
[56] M. Zhang, J. Gao, Q. Guo, X. Zhang, W. Zhang, Changes of respiratory syncytial virus infection in children before and after the COVID-19 pandemic in Henan, China. J Infect. 86 (2) (2023) 154–225.
[57] L. Li, R. Jia, Y. Zhang, H. Sun, J. Ma, Changes of parainfluenza virus infection in children before and after the COVID-19 pandemic in Henan, China. J Infect. 86 (5) (2023) 504–507.
[58] S. Karppinen, L. Toivonen, L. Schuez-Havupalo, M. Waris, V. Peltola, Interference between respiratory syncytial virus and rhinovirus in respiratory tract infections in children, Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis. 22 (2) (2016) 208.e1–208.e6.
[59] R.M. Greer, P. McErlean, K.E. Arden, C.E. Faux, A. Nitsche, S.B. Lambert, et al., Do rhinoviruses reduce the probability of viral co-detection during acute respiratory tract infections? J. Clin. Virol. Off. Publ. Pan. Am. Soc. Clin. Virol. 45 (1) (2009) 10–15.
[60] N.B. Achten, P. Wu, L. Bont, M.O. Blanken, T. Gebretsadik, J.D. Chappell, et al., Interference between respiratory syncytial virus and human rhinovirus infection in infancy, J Infect Dis 215 (7) (2017) 1102–1106.
[61] K. Dee, D.M. Goldfarb, J. Haney, J.A.R. Amat, V. Herder, M. Stewart, et al., Human rhinovirus infection blocks severe acute respiratory syndrome coronavirus 2 replication within the respiratory epithelium: implications for COVID-19 epidemiology, J. Infect. Dis. 224 (1) (2021) 31–38.
[62] M. Moriyama, W.J. Hugentobler, A. Iwasaki, Seasonality of respiratory viral infections, Annu. Rev. Virol. 7 (1) (2020) 83–101.