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Detection of Human Rhinovirus and Human Parainfluenza Virus among Young Children in Three Hospitals in Cambodia, 2020-2021

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ABSTRACT

Introduction

Human Rhinovirus (HRV) and Human Parainfluenza virus (HPIV) are the most common viral infections in the respiratory tract. HRV consists of three types: HRV-A, HRV-B and HRV-C, and HPIVs consist of four types: HPIV-1, HPIV-2, HPIV-3 and HPIV-4. Over 90% of the children aged two years had experienced at least one HRV infection, and 13% were infected with HPIV. This study aimed to determine the prevalence of HRV and HPIV among children under five years of age who had severe acute respiratory syndrome and were hospitalized.

Methods

This study used data from the Cambodian National Severe Acute Respiratory Infection (SARI) surveillance 2020--2021 using real-time RT–PCR to detect the HRV and HPIV on 888 respiratory swabs from Angkor Hospital for Children, National Pediatric Hospital, and Kirivong Referral Hospital (RH) among hospitalized children aged under 5 years between January 2020 and December 2021. We used Stata V16 for the data analysis, in which descriptive analysis was used to describe the frequency and percentage of HRV and HPIV infections and patient characteristics. The chi-square test was used to determine the associations between sociodemographic characteristics and HRV and HPIV positivity.

Results

More than sixty percent of the samples (62%) were boys. Among the total samples, 41.8% were from Angkor Hospital for Children, 33.1% were from National Pediatric Hospital, and 25.1% were from Kirivong RH. Closer to one-fifth of the total samples were confirmed to be positive for HRV infection (18.7%), followed by HPIV (3.2%) and coinfection between HRV and HPIV (1.0%). The HRV positive cases were significantly greater at the NPH site (22.1%) than at the Kirivong RH (21.5%) and AHC (14.3%) sites (P = 0.017). The number of HRV cases was the highest among those aged 13–24 months (30.1%), with P ≤0.001. The prevalence of HPIV slightly varied by site, sex and age group but was not significantly different.

Conclusion

This study revealed that acute respiratory tract infections, especially HRVs, are common among children under five years of age, followed by HPIVs. Coinfections involving HRV and HPIV were also observed, although the results were not significant. The spread of HRV has been observed year-round. Continued surveillance for diverse causes of pediatric respiratory illness could inform disease control and prevention program, including rollout of vaccination for preventable diseases.

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Keywords: HRV, HPIV, Polymerase chain reaction, Real-time RT–PCR and Severe acute respiratory infection.

Introduction

Human Rhinovirus (HRV) and Human Parainfluenza virus (HPIV) are the most common viral infections in the respiratory tract and are among the leading causes of morbidity and mortality worldwide in young children under the age of five (1). There are three HRV species: HRV-A, HRV-B, and HRV-C (2). HRV is an RNA virus that causes more than 50% of upper respiratory tract infections in humans worldwide (3). Infection with these HRVs can manifest as viral bronchiolitis in infants and is commonly associated with wheezing in children aged 1–2 years. Over 90% of children aged two years have experienced at least one HRV infection (3, 4).

HPIV is also a significant cause of acute respiratory illness in young children. There are four commonly recognized serotypes of HPIV: HPIV-1, HPIV-2, HPIV-3, and HPIV-4. In studies in Thailand and Gambia, HPIV was identified in 9.0% and 11.5% of hospitalized pediatric patients under 5 years of age, respectively (5, 6). The clinical manifestations of HPIV range from mild upper respiratory tract infection to wheezing and severe lower respiratory tract infections requiring mechanical ventilation.

Little is known about the circulation of HRV and HPIV viruses in remote populations throughout Cambodia, where the prevalence of HRV is 6% and that of HPIV is 3% (7). In the context of Cambodia, where the healthcare system may face challenges such as limited resources, understanding the prevalence, genetic diversity, and clinical impact of HRV and HPIV is crucial. This study to some extent could provide aid in the development of targeted public health interventions, improve clinical management, and potentially guide vaccine development.

Therefore, this study aimed to investigate the prevalence and distribution of HRV and HPIV infections among young children in three hospitals in Cambodia from 2020--2021 via polymerase chain reaction (PCR) via molecular techniques. These findings could be valuable data for developing public health strategies for managing and preventing respiratory infections in young children as well as for obtaining a global understanding of HRV and HPIV epidemiology in the country context.

Methods

A. Data Source

This study used samples collected through Cambodian National Severe Acute Respiratory Infection (SARI) surveillance from January 2020 to December 2021 among hospitalized children from three different hospitals: the National Pediatric Hospital (NPH) located in Phnom Penh, Angkor Hospital for Children (AHC) in Siem Reap, and Kirivong Referral Hospital (RH) in Takeo Province.

The Cambodian SARI surveillance consisted of 9 sentinel sites: NPH, Khmer-Soviet Friendship Hospital (KSFH), Cambodia-China Friendship Preah Kossamak Hospital (CCFH), Chey Chum Neah Hospital (Kandal), AHC (Siem Reap), Kampong Cham RH, Kirivong RH (Takeo), Kampot RH, and Svay Rieng RH.

To be eligible, participants included in SARI surveillance needed to have fever with an axillary temperature ≥ 38°C, cough or sore throat, and dyspnea and needed hospital admission. Nasopharyngeal and oropharyngeal swabs were collected from participants and transported to a central laboratory, the National Public Health Laboratory at the National Institute of Public Health (NIPH) in Phnom Penh, for processing.

B. Laboratory Management

The total sample of 888 hospitalized patients with nasopharyngeal and oropharyngeal swabs were randomly selected and tested from children aged less than five years admitted to hospitals with SARIs to determine the prevalence of HRV and HPIV virus infections. Informed consent was obtained from the legal guardians of the hospitalized children prior to enrolment.

Nucleic Acid Extraction

Nasopharyngeal and oropharyngeal samples were stored in viral transport media and transported to the molecular and virology laboratory of the NIPH at 4°C in a temperature-monitored transport box. Ribonucleic acid (RNA) was extracted from the samples via the QIAamp Viral RNA Extraction Mini Kit (Cat # 52906; Qiagen, Germany).

Real-Time Reverse Transcription Polymerase Chain Reaction

Real-time reverse transcription polymerase chain reactions (rRT–PCRs) were performed on Bio-Rad CFX96 and CFX96 Opus Touch real-time PCR detection system instruments. For HRV and HPIV, the SuperScript III Platinum One-Step qRT–PCR Kit (Cat # 11732--020,

Invitrogen) was used with specific oligonucleotide primers and probes for a single plex under the following conditions: reverse transcription at 50°C for 15 minutes, initial PCR denaturation at 95°C for 2 minutes, 40 cycles of denaturation at 95°C for 15 seconds, and annealing at 60°C for 30 seconds **(Table 1).**

Fluorescence data were collected during the 60°C incubation step. For the influenza and SARS-CoV-2 multiplex assay, the Quantabio qScript 1-Step Virus ToughMix Kit (Cat # 95131-02K) was used with the United State-Centers for Disease Control (US-CDC) oligonucleotide primers and probes and with conditions of reverse transcription at 50°C for 10 minutes, Taq inhibitor inactivation at 95°C for 1 minute, and PCR amplification at 45 cycles of 95°C for 5 seconds and 60°C for 30 seconds. Master mix failure and PCR contamination were monitored by adding a no-template negative control (UltraPure™ DNase/RNase-Free Distilled Water, catalog # 10977015) and a positive control comprising complementary deoxyribonucleic acid (cDNA) of the target viruses in each running batch.

Table 1: Primers and Probes for rRT–PCR *(8)*

Test	Primers and Probes	Sequence (5'>3')
Human Rhinovirus	HRV-F	TGGACAGGGTGTGAAGAGC
	HRV-R	CAAAGTAGTCGGTCCCATCC
	HRV-Probe	VIC-TCCTCCGGCCCCTGAATG-TAMRA
Human Parainfluenza Virus-1	HPIV-1 F	GTGATTTAAACCCGGTAATTTCTCA
	HPIV-1 R	CCTTGTTCCTGCAGCTATTACAGA
	HPIV-1 Probe	FAM-ACCTATGACATCAACGAC-MGBNFQ
Human Parainfluenza Virus-2	HPIV-2 F	ATGAAAACCATTTACCTAAGTGATGGA
	HPIV-2 R	CCTCCYGGTATRGCAGTGACTGAAC
	HPIV-2 Probe	VIC-TCAATCGCAAAAGC-MGBNFQ
Human Parainfluenza Virus-3	HPIV-3 F	CCAGGGATATAYTAYAAAGGCAAAA
	HPIV-3 R	CCGGGRCACCCAGTTGTG
	HPIV-3 Probe	FAM-TGGRTGTTCAAGACCTCCATAYCCGAGAAA-BHQ1
Human Parainfluenza Virus-4	HPIV-4 F	CAGAYAACATCAATCGCCTTACAAA
	HPIV-4 R	TGTACCTATGACTGCCCCAAARA
	HPIV-4 Probe	CY5-CCMATCACAAGCTCAGAAATYCAAAGTCGT-BHQ2
Influenza A Viruses	InfA-F-1	CAAGACCAATCYTGTCACCTCTGAC
	InfA-F-2	CAAGACCAATYCTGTCACCTYTGAC
	InfA-R-1	GCATTYTGGACAAAVCGTCTACG
	InfA-R-2	GCATTTTGGATA AAGCGTCTACG
	InfA-Probe	FAM-TGCAGTCCT/ZEN/CGCTCACTGGGCACG-3IABkFQ
Influenza B Viruses	InfB-F	TCCTCA AYTCACTCTTCGAGCG
	InfB-R	CGGTGCTCTTGACCA AATTGG
	InfB-Probe	YakYel-CCAATTCGA/ZEN/GCAGCTGAAACTGCGGTG-3IABkFQ
SARS-CoV-2 Virus	SC2-F	CTGCAGATTTGGATGATTTCTCC
	SC2-R	CCTTGTGTGGTCTGCATGAGTTTAG
	SC2-Probe	TexRd-XN/ATTGCAACA/TAO/ATCCATGAGCAGTGCTGA CTC-3IAbRQSp

C. Data Management and Analysis

The SARI data were entered into the National Public Health Lab SARI Surveillance Management System and imported into STATA V.16 (College Station, Texas, USA) for analysis. We restricted our testing to the period from January 2020--December 2021, with a total sample of 888. Descriptive statistics, including frequency, percentage, mean (SD), and median (IQR), were used to describe the sociodemographic characteristics of the study participants. We calculated the frequency of SARI patient characteristics and the proportion of PCR-positive cases. The chi-square test was used to assess the associations between sociodemographic characteristics and HRV and HPIV positivity.

Results

A. Sociodemographic characteristics of the participants

Of the total samples, 371 (41.8%) samples were from AHC, 294 (33.1%) were from NPH, and 223 (25.1%) were from Kirivong RH **(Table 2)**. Overall, 551 (62.1%) were boys. The median age of the patients was 11 months (range from 9 days to 5 years), and the ages of most of the participants ranged from 0 to 6 months: 334 (37.6%). The prevalence of HRV was 166, 18.7%, followed by HPIV (28, 3.2%) and a few cases of coinfection (9, 1.0%) with HRV and HPIV.

Table 2: Participants characteristics

Variables		N = 888	
		Freq.	%
Sites			
	AHC-SRP	371	41.78
	NPH-PNP	294	33.11
	Kirivong RH	223	25.11
Gender			
	Boys	551	62.05
	Girls	337	37.95
Age in months			
	Mean age (SD)	14 (13.94)	
	Median age (IQR)	11 (0.3 - 60)	
Aged group in months			
	00 – 06	334	**37.61**
	07 – 12	173	19.48
	13 – 24	216	24.32
	25 – 36	92	10.36
	37 – 48	49	5.52
	49 – 60	24	2.70
Viral infection			
	HRVs	166	18.69
	HPIVs	28	3.15
	HRV & HPIV	9	1.01

B. Distribution of HRV- and HPIV-positive patients

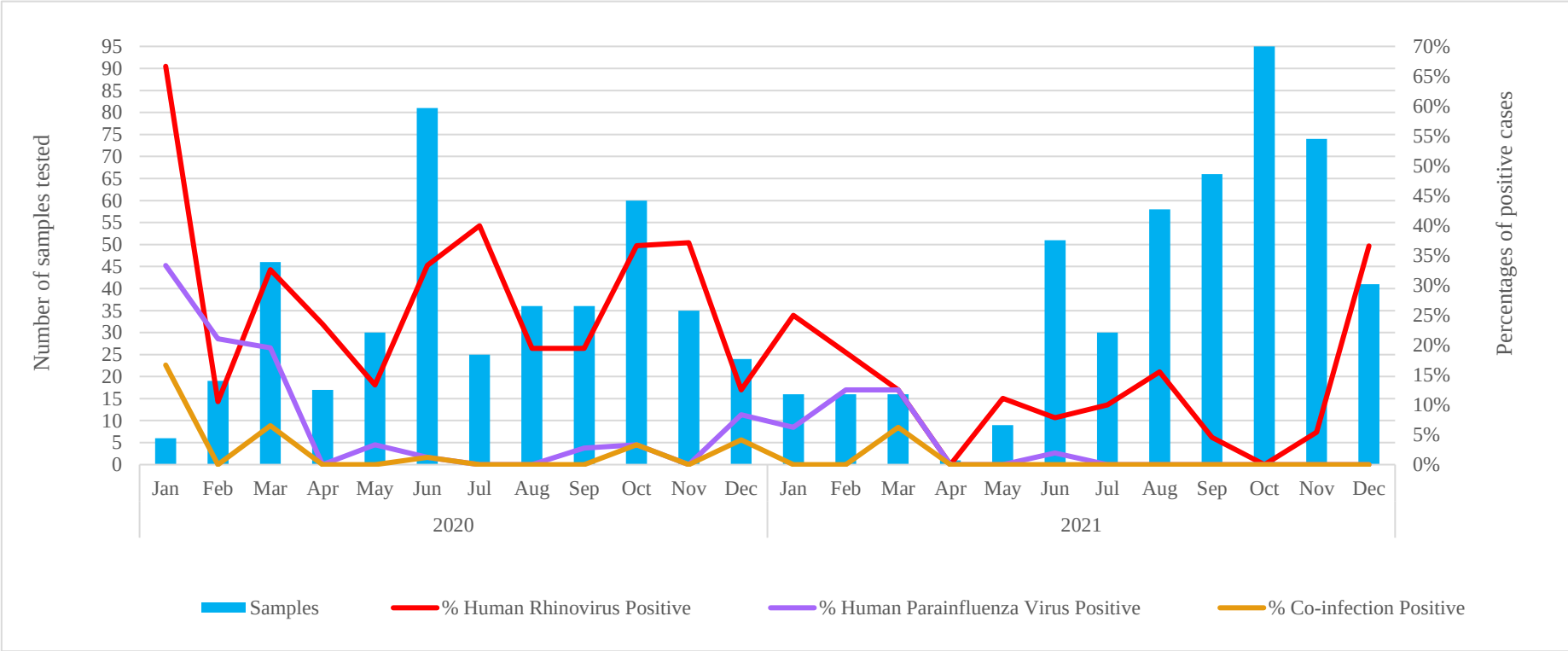
As shown in **Table 3**, the HRVs from the NPH site (65/294, 22.11%, P = 0.017) were greater than those from Kirivong RH (48/223, 21.52%) and AHC (53/371, 14.29%). The HRVs were slightly greater in boys (19.24%) than in girls (17.8%) and in the 13–24 month-age group (65/216, 30.1%) than in the other age groups (P ≤0.001). Furthermore, HPIV prevalence was also higher in Kirivong RH (4.93%) than in NPH (3.06%) and AHC (2.16%). Most of the HPIV cases involved girls (4.15%) and children aged 7–12 months (6.36%). Additionally, positive cases of coinfection between HRV and HPIV were observed, with 2.24% in Kirivong RH and 1.36% in NPH. Most coinfected patients were girls (1.48%) aged 13–24 months (1.85%).

Table 3: Distribution of HRV, HPIV, and combined HRV & HPIV by patient characteristics

Characteristics	Total sample	HRV			HPIV			HRV & HPIV		
		Positive	%	P value	Positive	%	P value	Positive	%	P value
Sites										
Angkor Hospital for Children	371	53	14.29	**0.017**	8	2.16	0.171	-	-	
National Pediatric Hospital	294	**65**	**22.11**		9	3.06		4	1.36	
Kirivong Referral Hospital	223	48	21.52		**11**	**4.93**		**5**	**2.24**	
Sex										
Boys	551	**106**	**19.24**	0.595	14	2.54	0.182	4	0.73	0.274
Girls	337	60	17.80		**14**	**4.15**		**5**	**1.48**	
Age group, month										
00 - 06	334	37	11.08	**≤ 0.001**	5	1.50	0.111	3	0.90	
07 - 12	173	33	19.08		**11**	**6.36**		1	0.58	
13 - 24	216	**65**	**30.09**		6	2.78		**4**	**1.85**	
25 - 36	92	19	20.65		3	3.26		1	1.09	
37 - 48	49	10	20.41		2	4.08		-	-	
49 - 60	24	2	8.33		1	4.17		-	-	

Figure 1 shows the HRV and HPIV samples tested from January 2020 to December 2021. Compared with that of HPIV, the trend of HRV infection shows a high peak among children that occurs almost every month of the year. For example, in January (67% and 25%), February (11% and 19%), March (33% and 13%), April (24%), May (13% and 11%), June (33% and 8%), July (40% and 10%), August (19% and 16%), September (19% and 5%), October (37%), November (37% and 5%) and December (13% and 37%). The monthly trend of HPIV was observed in a few months of the year, including January (33% and 6%), February (21% and 13%), March (20% and 13%), May (3%), June (1% and 2%), September (3%), October (3%) and December (8%). The incidence of coinfection with HRV-HPIV decreased over time from 2020–2021.

Figure 1: Total samples tested, and positive cases reported by month and year, 2020–2021.



Discussion

Overall, the HRV infection was mostly common (18.7%), followed by HPIV (3.2%). These results are lower than those of studies conducted in Queensland, Australia, for respiratory virus detection, in which the prevalence of HRV was 57.4%, with 686,199 samples tested (9). The prevalence of HPIV in our study was similar to that reported in a study conducted in China from 2020--2021 (10, 11). A peak of cases was observed between April and December 2021, which did not correspond to either of the two studied viruses, and we considered that other viruses may have circulated during that time. We suspect that other respiratory viruses may have contributed to these unexplained cases. In addition, we found that those aged between 13 and 24 months were significantly infected with HRV, which was comparable with the findings of a study of the impact of rhinovirus infection in children by Silvia Vandini (3).

We also detected coinfection between HRV and HPIV (3.2%), which was compatible with the findings of Lai et al. and Sreenath et al. (12, 13); however, this finding could not describe the severity of the illness. HRV was detected throughout the year, which is similar to the findings of Dupouey et al. (4). HPIV was detected within a few months of the year, which is almost identical to the results of the study by Morgan et al. (6). We did not find appreciable seasonality of HRV or HPIV during the study period since the cases occurred mostly every season. This is in keeping with pre-pandemic reports from Vietnam, in which the number of infections peaked from December to February (14), and from Malaysia, the countries' wet seasons but no appreciable seasonality of HRV (15). The peak number of cases was similar to that in Vietnam and Malaysia from December--February, but a confirmed study is needed since there was no evidence of cases during the wet season.

Conclusion

The study highlighted significant insights into respiratory infections among children in Cambodia, with the common HRV infection followed by HPIV, particularly among children aged 13 to 24 months. The spread of HRV has been observed year-round, where a decreased peak of HPIV has been observed between April-December, suggesting that other contributing factors may play a role, such as the circulation of another respiratory virus.

The valuable data obtained from this research can significantly inform public health strategies aimed at managing and preventing respiratory infections in this vulnerable population. By understanding the patterns of these viral infections, healthcare professionals can tailor interventions and improve outcomes for young children. Continued surveillance for diverse causes of pediatric respiratory illness could inform disease control and prevention program, including rollout of vaccination for preventable diseases.

Limitations

This study was based on Cambodia SARI surveillance data. Therefore, clinical data such as information on disease severity and outcomes could not be obtained. Testing was performed for a small panel of respiratory viruses and may have led to missed cases of pathogens for which methods of detection were not available. The available primer and probe reagents for subtypes of HRV and HPIV are limited. The number of samples used for testing in April was very low, which may have led to low virus detection.

Ethics statement

Ethical approval was obtained from Cambodia's National Ethics Committee for Health Research (Ref. # 209 NECHR). Informed consent was obtained from the legal guardians of the participating children prior to enrolment.

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