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Detection of respiratory viruses in gargle specimens of healthy children

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ABSTRACT

Background: Respiratory tract viral infection is one of the most common and important diseases in children. Polymerase chain reaction (PCR) tests are often used to detect viruses in samples, it is difficult to interpret the clinical significance of PCR positivity, which may reflect a past, imminent or active asymptomatic infection due to their high sensitivity. Although single respiratory viruses have been detected in samples from children with symptoms, other respiratory viruses can also be detected simultaneously. However, the clinical importance of these findings for the symptoms is not known.
Objectives: To investigate the prevalence of respiratory viruses among children without any symptoms such as acute respiratory illness and/or fever.
Study design: From week twenty-five 2013 to week twenty-six 2014, gargle samples were collected from children once a week and these samples were subjected to real-time PCR to detect respiratory viruses. On each sampling day, we asked the parents about their children's health condition.
Results: Among the 286 samples collected, 200 were from asymptomatic children. In the asymptomatic condition, human parechovirus, adenovirus, enterovirus, rhinovirus, coronavirus 229E and HKU1 were observed in 45 episodes. In samples from symptomatic children, parainfluenza viruses, respiratory syncytial virus and coronavirus OC43 were detected in addition to those mentioned above.
Conclusions: Various viruses of different species were detected in the specimens from the children regardless of their health status. It might be speculated that host factors such as the function of the immune system influence the clinical outcome of the infection. However, this needs to be studied further.

1. Background

Respiratory tract viral infection is one of the most common and important disease conditions in children. Recently, PCR based assays have made it possible for novel viruses to be discovered, leading to appraisal of the clinical impacts of these viruses and several other well-known respiratory viruses [1–4]. Some of these viruses are detected alone in specimens from patients with respiratory symptoms (sometimes in those of inpatients) but their pathogenicity is not clear because they are detected simultaneously with other viruses in many cases [5–7]. As a result, the clinical importance of these findings for the symptoms is not known.

2. Objectives

In this study, we investigated how often and what respiratory viruses were detected in specimens from asymptomatic children. Gargle specimens (obtained by rinsing the throat with distilled water) were collected from children once a week and the samples were subjected to two-step real-time PCR to detect respiratory viruses. Singleplex real-time PCR procedures were employed for detection of the following 15 respiratory viral pathogens: parainfluenza viruses (PIV) 1–4, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), enterovirus (EV)/rhinovirus (RV), human bocavirus (hBoV), human parechovirus (hPeV), adenovirus (AdV), and human coronaviruses (hCoV) OC43, NL63, 229E, and HKU-1 (Table 1), and one-step real-time reverse transcription (RT)–PCR was used for detection of influenza viruses (FluV) A and B (Table 1).

Abbreviations: PCR, polymerase chain reaction; PIV, parainfluenza virus; RSV, respiratory syncytial virus; hMPV, human metapneumovirus; EV, enterovirus; RV, rhinovirus; RVA, rhinovirus genogroup A; RVB, rhinovirus genogroup B; RVC, rhinovirus genogroup C; hBoV, human bocavirus; hPeV, human parechovirus; AdV, adenovirus; hCoV, human coronavirus; FluV, influenza virus; RT, reverse transcription.

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Table 1
Primers and probes used in this study.

Virus	Target	Product size(bp)	Specific primers and probes	Detection limit (copy/uL)	Reference
PIV1	HN	135	Antisense 5' GTCCTTCCTGCTGGTGTGTTAAT 3'	6.55×10^2	
			Sense 5' CCAACCTACAAGGCAACAACATC 3'		[27]
			Probe 5' (FAM)CAAACGATGGCTGAAAA(TAMRA) 3'		
PIV3	HN	161	Antisense 5' TTGTTATAGTGTGTAATGCAGCTCGT 3'	5.30×10^2	
			Sense 5' GGGAGCATTGTGTCATCTGTCA 3'		[27]
			Probe 5' (FAM)CCCAGTCATAACTTACTC(TAMRA) 3'		
PIV2	NP	65	Antisense 5' TCYTCAGCTAATGCTTCRAARGC 3'	1.0×10^2	
			Sense 5' ATTCCAGATGCTCGATCAACTATG 3'		[28]
			Probe 5' (FAM)AGCACYTCTCCTCTGG(TAMRA) 3'		
PIV4	NP	123	Antisense 5' ATGTGGCCTGTAAGGAAAGCA 3'	1.0×10^1	
			Sense 5' CAAAYGATCCACAGCAAAGATTC 3'		[29]
			Probe 5' (FAM)GTATCATCATCTGCCAAATCGGCAATTAAACA(TAMRA) 3'		
RSV	F	89	Antisense 5' CGATTTTTATTGGATGCTGTACATTT 3'	2.22×10^2	
			Sense 5' AACAGATGTAAGCAGCTCCGTTATC 3'		[30]
			Probe 5' (FAM)TGCCATAGCATGACACAATGGCTCCT(TAMRA) 3'		
hPMV	M	152	Antisense 5' CATCAGCCYYATCWGTGTTTCTTAAAA 3'	2.47×10^2	
			Sense 5' GGCTCCATGCAAATATGAAGTG 3'		[31]
			Probe 5' (FAM)CTAACGAGTGTGCGCAAG(TAMRA) 3'		
EV/RV	5'NTR[b]	203	Antisense 5' GAAACACGGACACCCAAAGTAGT 3'	Echo 9.76×10	
			Sense 5' AGCCTGCGTGGCKGCC 3'	RVC 2.98×10^2	[32]
			Probe 5' (FAM) CTCCGGCCCCTGAATGYGGCTAA(TAMRA) 3'		
hBoV	NP-1	75	Antisense 5' TGGACTCCCTTTTCTTTTGTAGGA 3'	5.05×10^2	
			Sense 5' GCACAGCCACGTGACGAA 3'		[33]
			Probe 5' (FAM)TGAGCTCAGGGAATATGAAAGACAAGCATCG(TAMRA) 3'		
hCoV229E	NC	80	Antisense 5' TCTTTTCCACCGTGGCTTTT 3'	1.0×10^2	
			Sense 5' CTGCCAAGAGTCTTGCTCGTT 3'		[28]
			Probe 5' (FAM)AGAACAAAAGCATGAAATG(TAMRA) 3'		
hCoVNL63	NC	61	Antisense 5' CGAGGACCAAAGCACTGAATAA 3'	1.17×10^2	
			Sense 5' AACCTCGTTGGAAGCGTGTT 3'		[28]
			Probe 5' (FAM)ATTTTCCTCTCTGGTAG(TAMRA) 3'		
hCoVOC43	NC	67	Antisense 5' GCTGAGGTTTAGTGGCATCCTT 3'	2.19×10^2	
			Sense 5' GACATGGCTGATCAAATTGCTAGT 3'		[28]
			Probe 5' (FAM)TCTGGCAAAACTTGG(TAMRA) 3'		
hCoV HKU	ORF 1a/b	61	Antisense 5' CATTCATTCGCAAGGCGATA 3'	1.11×10^2	
			Sense 5' CCCGCAAACATGAATTTTGTT 3'		[28]
			Probe 5' (FAM)AATCTATCACCATGTGAA (TAMRA) 3'		
hPeV	5'NTR	194	Antisense 5' GGCCCCWGRTCAGATCCAYAGT 3′	1.0×10^2	
			Sense 5' GTAACASWWGCCTCTGGGSCCAAAAG 3′		[34]
			Probe 5'(FAM)CCTRYGGGTACCTYCWGGGCATCCTTC(TAMRA) 3′		
AdV(ACDF)	Hexon	85	Antisense 5' AAACTTGTTATTCAGGCTGAAGTACGT3'	1.0×10^2	
			Sense 5' CCAGGACGCCTCGGAGTA 3'		[35]
			Probe 5' (FAM)AGTTTGCCCGCGCCACCG(TAMRA) 3'		
AdV(BE)	Hexon	81	Antisense 5' CTTGTTCCCCAGACTGAAGTAGGT 3'	1.0×10^2	
			Sense 5' GGACAGGACGCTTCGGAGTA 3'		[35]
			Probe 5' (FAM)CAGTTCGCCCGYGCMACAG(TAMRA) 3'		
FluV typeA	MP	149	Antisense 5' TGACAGRATYGGTCTTGTCTTTAGCCAYTCCA	7.5[a]	
			Sense 5' CCMAGGTCGAAACGTAYGTTCTCTCTATC		[36]
			Probe 5' (FAM)ATYTCGGCTTTGAGGGGGCCTG(MGB) 3'		
FluV AH1pdm09	HA	187	Antisense 5' TGTTTCCACAATGTARGACCAT	6.8[a]	
			Sense 5' AGAAAAGAATGTAACAGTAACACACTCTGT		[36]
			Probe 5' (FAM)CAGCCAGCAATRTTRCATTTACC(MGB) 3'		
FluV AH3	HA	178	Antisense 5'GTCATTGGGRATGCTTCCATTTGG	7.1[a]	
			Sense 5' CTATTGGACAATAGTAAAACCGGGRGA		[36]
			Probe 5' (FAM)AAGTAACCCCKAGGAGCAATTAG(MGB) 3'		
FluV B	NS	105	Antisense 5'GTKTAGGCGGTCTTGACCAG	8.2[a]	
			Sense 5' GGAGCAACCAATGCCAC		[37]
			Probe 5' (FAM)ATAAACTTTGAAGCAGGAAT(MGB) 3'		

[a] From reference data.
[b] NTR: non translated region.

3. Study design

3.1. Subjects

Twelve children aged 3–10 years old were enrolled. From week twenty-five 2013 to week twenty-six 2014, throat gargle samples were obtained from the children once a week. Their parents noted the existence of respiratory symptoms (cough, sore throat or nasal mucus) and systemic symptoms (fever or rash) at the time of sampling. Written informed consent was obtained from the parents.

3.2. Molecular analysis

Nucleic acids were extracted from 200 μL specimens using the Magtration System with a MagDEA viral DNA/RNA 200 kit (Precision System Science Co., Ltd., Chiba, Japan) as 50 μL of elution volume. RT reactions were performed using a ReverTra Ace qPCR RT kit (TOYOBO Co., Ltd., Osaka, Japan) following the manufacturer's instructions. The cDNA was then amplified using Realtime PCR Master Mix (TOYOBO) with a total volume of 25 μL. Each sample was amplified containing primers and probes specific for each of the targets as described in Table 1 [27–37]. The sensitivity of each of the