Supplementary Materials

Article Title: A decade of human metapneumovirus in hospitalized children with acute respiratory infection: Molecular epidemiology in central Vietnam, 2007-2017

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Subgroup, genotype, and sublineage classification of hMPV

HMPV subgroup A and B were further classified into genotypes A1, A2, B1, and B2 based on genetic differences in a previous report [1]. A recent study reported that hMPV A2 can be further categorized into several branches, A2a, A2b, and A2c, according to F gene phylogenetic analysis [2]. Furthermore, variants with 180- or 111-nucleotide duplication in the G gene were found to be included in the A2c branch in phylogenetic analysis of the F and G gene [3–5]. We therefore differentiated hMPV A2a, A2b, and A2c as sublineages of genotype A2 in our analysis. To classify the obtained sequences into these genotypes and sublineages, we conducted a phylogenetic analysis with reference sequences. The hMPV F gene sequences were aligned with the Clustal W algorithm implemented within *msa* package [6] in R version 4.1.2 [7]. Accession numbers in GeneBank of the representative reference sequences used for classification were AF371337 (A1), AB503857 (A2a), AY530095 (A2b), GQ153651 (A2c), AY525843 (B1), and FJ168778 (B2) [8]. Accession numbers in GeneBank of our sequences for representatives in genotypes and sublineages are OP947591–OP947594. Samples that failed to be amplified were classified as genotype undetermined.

References

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Supplementary Table 1. Clinical features of patients with hMPV

	hMPV vs RSV			hMPV vs Non-RSV		
		Supplementary Table 1				
Characteristics	**hMPV, N = 278**	**RSV, N = 1,671**	**p-value**[a]	**hMPV, N = 278**	**Non-RSV, N = 6,873**	**p-value**[b]
Age Month (Median (IQR))	21.0 (12.7, 32.5)	13.9 (6.8, 22.6)	<0.001	21.0 (12.7, 32.5)	17.9 (9.6, 30.1)	0.011
Age Group (n (%))			<0.001			<0.001
1-5mo	13 (4.7%)	24 (1.4%)		13 (4.7%)	578 (8.4%)	
6-11mo	29 (10.4%)	363 (21.7%)		29 (10.4%)	926 (13.5%)	
12-23mo	93 (33.5%)	577 (34.5%)		93 (33.5%)	2,252 (32.8%)	
24-35mo	69 (24.8%)	252 (15.1%)		69 (24.8%)	1,111 (16.2%)	
36-47mo	22 (7.9%)	70 (4.2%)		22 (7.9%)	485 (7.1%)	
48-59mo	16 (5.8%)	18 (1.1%)		16 (5.8%)	242 (3.5%)	
≧60mo	36 (12.9%)	367 (22.0%)		36 (12.9%)	1,279 (18.6%)	
Sex (n (%))			0.2			0.2
Male	152 (54.7%)	980 (58.6%)		152 (54.7%)	4,023 (58.5%)	
Female	126 (45.3%)	691 (41.4%)		126 (45.3%)	2,850 (41.5%)	
Cough (n (%))	277 (99.6%)	1,667 (99.8%)	0.5	277 (99.6%)	6,847 (99.6%)	>0.9
Difficulty of Breathing (n (%))	98 (35.3%)	579 (34.6%)	0.8	98 (35.3%)	1,729 (25.2%)	<0.001
Tachypnea (n (%))	201 (72.3%)	1,314 (78.6%)	0.019	201 (72.3%)	5,653 (82.2%)	<0.001
Chest Indrawing (n (%))	18 (6.5%)	134 (8.0%)	0.4	18 (6.5%)	299 (4.4%)	0.092
Stridor (n (%))	4 (1.4%)	17 (1.0%)	0.5	4 (1.4%)	111 (1.6%)	>0.9
Wheeze (n (%))	150 (54.0%)	935 (56.0%)	0.5	150 (54.0%)	3,017 (43.9%)	<0.001
Crackle (n (%))	52 (18.7%)	292 (17.5%)	0.6	52 (18.7%)	785 (11.4%)	<0.001
Clinical Pneumonia (n (%))			0.10			<0.001

	hMPV vs RSV			hMPV vs Non-RSV		
Characteristics	**hMPV**, N = 278	**RSV**, N = 1,671	**p-value**[a]	**hMPV**, N = 278	**Non-RSV**, N = 6,873	**p-value**[b]
No pneumonia	197 (70.9%)	1,261 (75.5%)		197 (70.9%)	5,559 (80.9%)	
Pneumonia	81 (29.1%)	410 (24.5%)		81 (29.1%)	1,314 (19.1%)	
Presence of Danger Sign (n (%))	9 (3.2%)	47 (2.8%)	0.7	9 (3.2%)	162 (2.4%)	0.3
Body Temperature (Mean (SD))	38.1 (0.8)	38.0 (0.8)	0.5	38.1 (0.8)	38.1 (0.9)	0.6
Duration of Hospitalization (days, Median (IQR))	5.0 (3.0, 6.0)	5.0 (4.0, 7.0)	0.10	5.0 (3.0, 6.0)	4.0 (3.0, 6.0)	<0.001
Onset to Hospitalization (days, Median (IQR))	2.0 (1.0, 4.0)	3.0 (2.0, 4.0)	0.042	2.0 (1.0, 4.0)	2.0 (1.0, 4.0)	0.8
Unknown	45	216		45	806	
Onset to Discharge (days, Median (IQR))	7 (5, 9)	8 (6, 10)	0.002	7.0 (5.0, 9.0)	7.0 (5.0, 10.0)	0.2
Unknown	45	216		45	806	

[a]Kruskal-Wallis rank sum test; Fisher's Exact Test for Count Data with simulated p-value (based on 2000 replicates); Pearson's Chi-squared test; Fisher's exact test; Wilcoxon rank sum test

[b]Kruskal-Wallis rank sum test; Pearson's Chi-squared test; Fisher's exact test; Wilcoxon rank sum test

Abbreviations: IQR, interquartile range; hMPV, human metapneumovirus-positive group, RSV: respiratory syncytial virus-positive group, Non-RSV: human metapneumovirus–respiratory syncytial virus double-negative group

Supplementary Table 2. Comparison between hMPV patients with or without co-detected virus

| Characteristics | Overall, N = 278 | hMPV with Co-detected Virus | | p-value[a] |
		No, N = 201	Yes, N = 77	
Age Month (Median (IQR))	21.0 (12.7, 32.5)	22.9 (12.7, 35.0)	18.9 (13.0, 26.8)	0.044
Age Group (n (%))				0.052
1-5mo	13 (4.7%)	11 (5.5%)	2 (2.6%)	
6-11mo	29 (10.4%)	20 (10.0%)	9 (11.7%)	
12-23mo	93 (33.5%)	56 (27.9%)	37 (48.1%)	
24-35mo	69 (24.8%)	53 (26.4%)	16 (20.8%)	
36-47mo	22 (7.9%)	19 (9.5%)	3 (3.9%)	
48-59mo	16 (5.8%)	14 (7.0%)	2 (2.6%)	
≧60mo	36 (12.9%)	28 (13.9%)	8 (10.4%)	
Sex (n (%))				<0.001
Male	152 (54.7%)	97 (48.3%)	55 (71.4%)	
Female	126 (45.3%)	104 (51.7%)	22 (28.6%)	
Cough (n (%))	277 (99.6%)	200 (99.5%)	77 (100.0%)	>0.9
Difficulty of Breathing (n (%))	98 (35.3%)	68 (33.8%)	30 (39.0%)	0.4
Tachypnea (n (%))	201 (72.3%)	150 (74.6%)	51 (66.2%)	0.2
Chest Indrawing (n (%))	18 (6.5%)	11 (5.5%)	7 (9.1%)	0.3
Stridor (n (%))	4 (1.4%)	3 (1.5%)	1 (1.3%)	>0.9
Wheeze (n (%))	150 (54.0%)	105 (52.2%)	45 (58.4%)	0.4
Crackle (n (%))	52 (18.7%)	38 (18.9%)	14 (18.2%)	0.9
Clinical Pneumonia (n (%))				0.10
No pneumonia	197 (70.9%)	148 (73.6%)	49 (63.6%)	
Pneumonia	81 (29.1%)	53 (26.4%)	28 (36.4%)	
Presence of Danger Sign (n (%))	9 (3.2%)	5 (2.5%)	4 (5.2%)	0.3

Supplementary Table 2

| Characteristics | Overall, N = 278 | hMPV with Co-detected Virus | | p-value[a] |
		No, N = 201	Yes, N = 77	
Body Temperature (Mean (SD))	38.1 (0.8)	38.0 (0.9)	38.2 (0.8)	0.2
Duration of Hospitalization (days, Median (IQR))	5.0 (3.0, 6.0)	5.0 (4.0, 6.0)	4.0 (3.0, 6.0)	0.022
Onset to Hospitalization (days, Median (IQR))	2.0 (1.0, 4.0)	2.0 (1.0, 3.0)	3.0 (1.0, 5.0)	0.2
Unknown	45	33	12	
Onset to Discharge (days, Median (IQR))	7.0 (5.0, 9.0)	7.0 (6.0, 9.0)	7.0 (5.0, 11.0)	0.8
Unknown	45	33	12	

[a]Kruskal-Wallis rank sum test; Fisher's Exact Test for Count Data with simulated p-value (based on 2000 replicates); Pearson's Chi-squared test; Fisher's exact test; Wilcoxon rank sum test

Abbreviations: IQR, interquartile range; hMPV, human metapneumovirus

Supplementary Table 3. Association between subgroup, genotypes, and sublineage and wheeze frequency adjusted for age months.

						Supplementary Table 3										
		A vs B						**A2 vs B1 vs B2**					**A2b vs A2c vs B1 vs B2**			
Characteristic	**N**	**Event N**	**OR[a]**	**95% CI[a]**	**p-value**	**N**	**Event N**	**OR[a]**	**95% CI[a]**	**p-value**	**N**	**Event N**	**OR[a]**	**95% CI[a]**	**p-value**
Age Months	145	77	1.00	0.98, 1.02	>0.9	145	77	1.00	0.98, 1.02	>0.9	145	77	1.00	0.98, 1.02	0.9
Subgroup															
A	106	60	—	—											
B	39	17	0.59	0.28, 1.24	0.2										
Genotype															
B1						26	11	—	—						
B2						13	6	1.17	0.30, 4.52	0.8					
A2						106	60	1.78	0.75, 4.34	0.2					
Sublineage															
B1											26	11	—	—	
B2											13	6	1.17	0.30, 4.51	0.8
A2c											76	37	1.29	0.52, 3.23	0.6
A2b											30	23	4.48	1.46, 14.9	**0.011**

[a]OR = Odds Ratio, CI = Confidence Interval
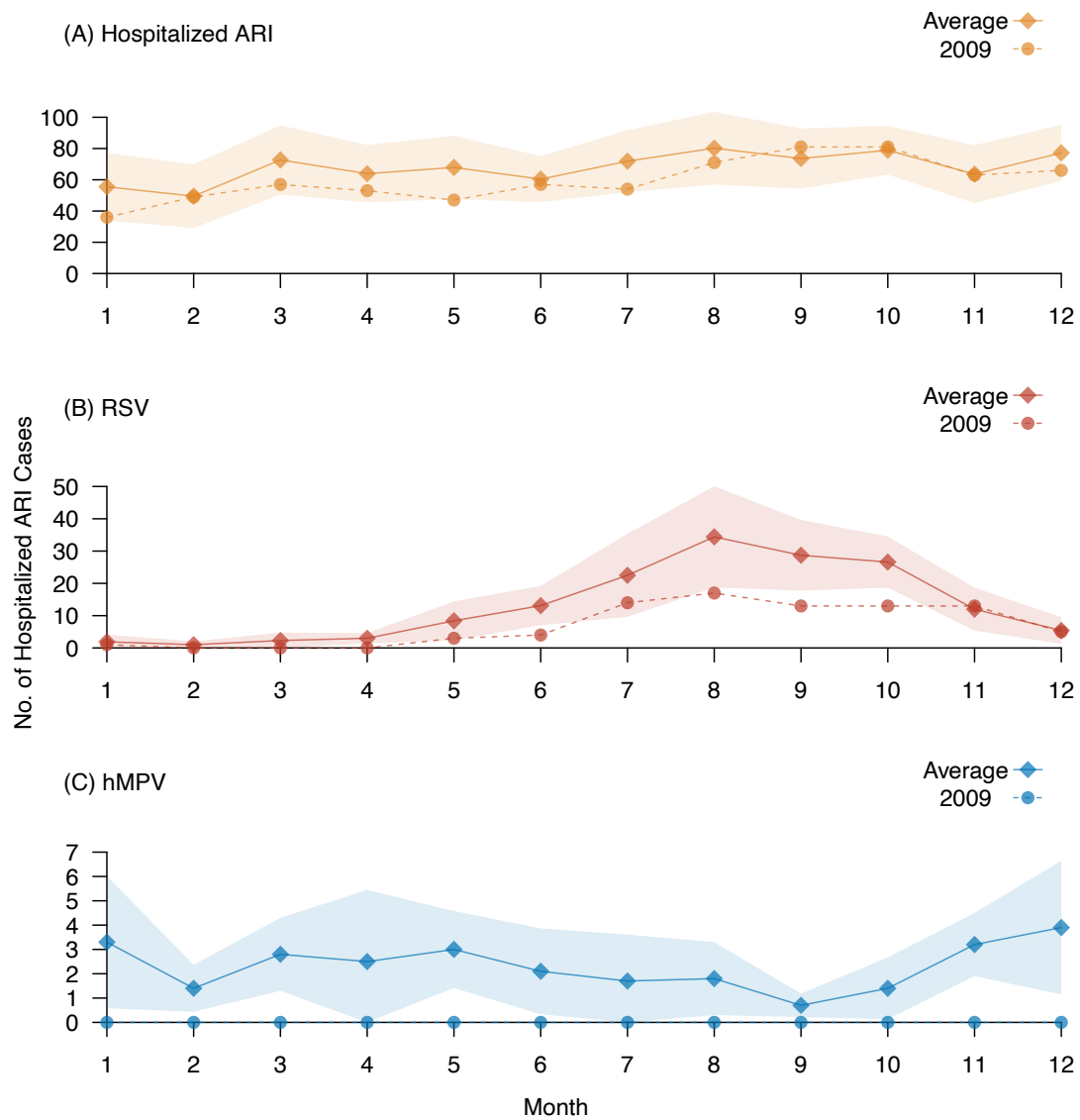
Supplementary Figure 1

The monthly number of cases for (A) hospitalized ARI, (B) RSV-positive, and (C) hMPV-positive. The solid lines with diamonds show the monthly average cases from 2007 to 2017 with the 95% confidence interval, except for 2009. The dashed lines with circles show the number of hospitalized ARI cases in 2009.