Original Article

Prevalence and Characteristics of Human Metapneumovirus Infection Among Hospitalized Children at High Risk for Severe Lower Respiratory Tract Infection

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Background. Human metapneumovirus (HMPV) is a significant cause of respiratory tract infections. Little is known about HMPV in children who are at high risk for lower respiratory tract infection (LRTI).
Methods. To determine the prevalence of HMPV in high-risk children and to identify HMPV risk factors, children ≤24 months with prematurity, chronic lung disease, and/or congenital cardiac disease who were hospitalized with LRTI were prospectively enrolled. Nasopharyngeal aspirates were tested for HMPV, respiratory syncytial virus (RSV), influenza A and B, and parainfluenza types 1–3. Demographics, medical history, and outcomes for those with HMPV and RSV were compared. A multivariate analysis was performed to determine HMPV risk factors.
Results. Over 4 years, 1126 eligible children were enrolled. Pathogens were identified in 61% of subjects. HMPV was identified in 9.0%, second to RSV (45%). Coinfection with HMPV and RSV occurred in <1% of subjects. Subjects infected with HMPV were older (8.2 vs 4.0 months, $P < .001$), were born more prematurely (27 vs 33 weeks, $P < .001$), and more commonly had chronic lung disease (59.3% vs 21.8%, $P < .001$) compared with subjects infected with RSV. In a multivariate analysis that compared children infected with HMPV to all others, increasing age and household exposure to children ages 6–12 were associated with an increased risk, whereas birth at older gestational age and exposure to children age >12 were associated with a decreased risk.
Conclusions. HMPV was detected in 9% of high-risk children who were hospitalized with lower respiratory tract disease, representing the second most common virus in this population. Compared with all other subjects (including RSV-infected), subjects infected with HMPV were older but were born more prematurely.

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Since its initial recognition in 2001 [1], human metapneumovirus (HMPV) has been identified as a common cause of acute upper respiratory tract infections, croup, bronchiolitis, and pneumonia in children [2–5]. Most studies suggest that HMPV infections peak in the winter–spring [5–9], occur in slightly older children [4, 7, 10], and may be less severe than respiratory syncytial virus (RSV) infections [9]. HMPV is ubiquitous, because >90% of children have serological evidence of prior HMPV infection by the age of 5–9 years [3]. Recurrent infections occur throughout life [5, 11], with an estimated 1–9% of adults infected each year [11, 12]. Most recurrent infections are asymptomatic, but symptomatic illness that can be severe has been described in the elderly, those with underlying pulmonary disease, and the immunocompromised [11, 12]. Risk factors described for severe disease in children vary but have included coinfection with RSV [13–15], prematurity [10, 13], asthma [7], and other underlying abnormalities [10, 16]. Prospective data regarding HMPV have been limited to studies conducted for only 1 or 2 years or from limited geographic areas [17, 18].

To assess the importance of and risk factors for HMPV in a high-risk population, we conducted a multicentered, international, multiyear, prospective study of young children with prematurity, chronic lung disease (CLD) of prematurity, and/or congenital heart disease (CHD) who were hospitalized with lower respiratory tract infections (LRTIs). The prevalence of LRTIs due to HMPV infection in this population was compared with the prevalence of other infections including RSV A and B, influenza A and B (INF A/B), parainfluenza types 1, 2, and 3 (PIV 1–3), and coinfections. In addition, the epidemiological and clinical characteristics of high-risk children with HMPV-associated LRTI requiring hospitalization were determined.

METHODS

Study Design

A prospective, multicenter, international study was conducted to determine the prevalence of HMPV in children ≤24 months of age and who were at high risk for hospitalization with severe respiratory disease during the fall to spring seasons in the northern and southern hemispheres. Subjects could be re-enrolled as long as entry criteria continued to be met. Enrollment occurred annually between October 1 and June 30 beginning in December 2002 through June 2006 at 24 sites in the northern hemisphere (United States, Canada, Italy, and The Netherlands) and between March 1 and November 30 beginning in April 2003 through November 2006 at 3 sites in the southern hemisphere (Australia). Local institutional review boards approved the study before enrollment of any subject.

Subjects

Eligibility included children ≤12 months of age who were born prematurely (<36 weeks gestation), children ≤24 months of age with hemodynamically significant CHD, and/or CLD of prematurity who required medical intervention (eg, supplemental oxygen, corticosteroids, bronchodilators, or diuretics) within the previous 6 months. Subjects with exposure or infection with human immunodeficiency virus were excluded.

Parents or legal guardians of subjects diagnosed with acute LRTI (eg, bronchiolitis, bronchitis, pneumonia, or cardiac decompensation associated with respiratory infection) were asked to enroll the child within 2 days of hospital admission. Written informed consent was obtained before participation in the study.

Clinical Data Collection

The following were collected from the parent interview and medical records on prospective data collection forms: demographic data; medical, family, and social history; and use of palivizumab, RSV-immune globulin, ribavirin, inhaled corticosteroids, and immunosuppressive medications (eg, systemic corticosteroids) within 1 month preceding enrollment. Some of these data were not collected during the initial year of the study. No data on palivizumab use, besides the month before admission, were collected. Physical examination findings and details regarding the hospital course through discharge or study day 28, whichever came first, were collected.

Laboratory Methods

Nasopharyngeal wash aspirates (NPAs) or endotracheal aspirates were obtained from subjects on the day of study enrollment. For subjects requiring mechanical ventilation after study entry but ≤28 days from admission, NPAs and endotracheal aspirates were collected within 2 days of initiation of mechanical ventilation. Samples were stabilized in an equal volume of viral transport medium and kept refrigerated until snap frozen to –70°C or colder within 2 hours of collection.

All samples were evaluated for HMPV at Focus Diagnostics, Inc (Cypress, CA) by a real-time, reverse transcription-polymerase chain reaction (RT-PCR) assay using primer and probe sequences previously published as NL-N forward, NL-N reverse, and NL-N-probe [19]. Extraction of RNA from NPA and/or endotracheal samples was conducted with the QIAamp Viral RNA kit (QIAGEN Inc, Valencia, CA).

A multiplex RT-PCR enzyme hybridization assay (Hexaplex; Prodesse, Inc., Waukesha, WI) was used for rapid simultaneous detection of RSV A and B, INF A and B, and PIV 1–3 nucleic acid [20]. Extraction of viral nucleic acids was conducted using the High Pure Viral Nucleic Acid kit (Roche Applied Science, Indianapolis, IN). Samples with a negative RSV result were retested for RSV B with the TaqMan PCR method at Cogenics Inc. (Houston, TX) to optimize recovery [21].

Only subjects who met entry criteria and had a sample obtained were analyzed (Figure 1). Subjects with indeterminate viral testing results were excluded from the analysis population for that specific virus. Cases where HMPV was identified as the sole agent were designated as an HMPV infection, whereas cases in which HMPV was recovered with another virus were designated as HMPV coinfection. Prevalence calculations included both infections and coinfections.

Statistical Analysis

To determine sample size, a prevalence rate of 10% for HMPV LRTI was assumed, for which 1100 subjects would estimate the prevalence of LRTI with a precision <2.0%, providing an exact 95% confidence interval (CI) of 8.3%–11.9%.

Data were analyzed using SAS® System Version 9.1.3 (SAS Institute Inc, Cary, NC) in a UNIX environment. Categorical data were summarized by the number and percentage of subjects in each category. Continuous variables were summarized by descriptive statistics including median and range. Median values were compared using Wilcoxon rank test, proportions between 2 groups were compared using Fisher's exact test, and proportions between more than 2 groups were compared using χ^2 test. To adjust for multiple comparisons, results with a 2-tailed P value $\leq$.005 were considered statistically significant [22].

Logistic regression with stepwise selection (entry alpha = 0.2, exit alpha = 0.25) was used to determine predictors of HMPV infection when compared with all other subjects. Variables used in model selection included age (in months), gestational age, multiple birth, risk factors (premature, premature with CLD, and CHD), number of adults (18 years) in the household, and number of other children (<6, 6–12, >12–18 years) in the household. Palivizumab use at baseline was excluded as a variable because data regarding all prior use was not collected.

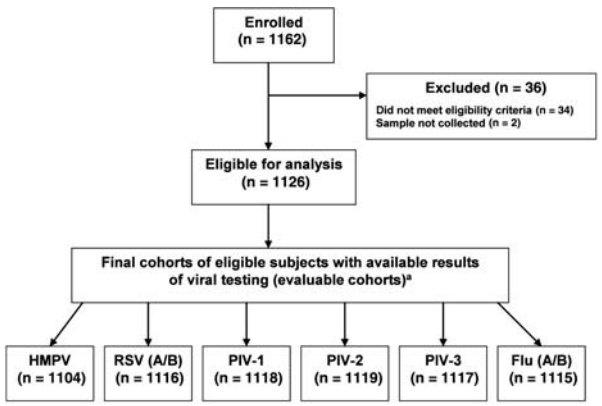

Figure 1. Flow of study participants. Abbreviations: HMPV, human metapneumovirus; PIV, parainfluenza; RSV, respiratory syncytial virus.
[a]Subjects with indeterminate results were excluded from analysis.

RESULTS

Of 1162 children enrolled, 1126 (97%) were analyzed (Figure 1). Eighty-six percent (968) of these subjects were enrolled in the northern hemisphere. Overall, 57.9% were male, 49.7% were white, 40.9% had a gestational age between 32 and <36 weeks, 37.7% received palivizumab within 1 month before enrollment, and 85.0% (763 of 898) had a previous neonatal intensive care unit admission. Underlying risk factors are presented in Table 1. Of the children with CHD, 20% of those $\leq$12 months of age and 24% of those >12–24 months of age had cyanotic CHD.

A specific virus was identified in 61.0% of eligible children (Table 2). Overall, HMPV was identified in 9.0%, second only to RSV (45.0%); PIV-3, INF A/B, and PIV-1 were identified in 4.0%, 4.0%, and 1.0%, respectively. The prevalence of HMPV was significantly higher in the northern hemisphere than in the southern hemisphere (10.0% vs 3.0%; P = .001), whereas PIV-3 was significantly higher in the southern hemisphere (8.0% vs 3.0%; P = .005). The prevalence of RSV was similar between the hemispheres (Table 2). The seasonality of the different viruses in each hemisphere is depicted in Figure 2. Coinfection with HMPV and RSV was rare (8 subjects, <1%), and clinical outcomes were similar to HMPV or RSV

Table 1. Baseline Demographics and Medical History of Subjects Eligible for Analysis (n = 1126)

	Premature (Without CHD)[a]			Any CHD[b]	
	Without CLD		With CLD		
	Age ≤6 Months[c] (n = 481)	Age >6–12 Months[c] (n = 64)	Age ≤24 Months[d] (n = 377)	Age ≤12 Months (n = 146)	Age >12–24 Months (n = 58)
Age (months), median (range)	2.60 (0.4–6.0)	8.75 (6.1–12.0)	9.30 (0.7–24.0)	5.45 (0.6–12.0)	15.80 (12.1–23.8)
Male, n (%)	285 (59.3)	35 (54.7)	232 (61.5)	67 (45.9)	33 (56.9)
Race/ethnicity, n (%)					
White/non-Hispanic	247 (51.4)	33 (51.6)	177 (46.9)	66 (45.2)	37 (63.8)
Black	97 (20.2)	10 (15.6)	76 (20.2)	12 (8.2)	3 (5.2)
Hispanic	91 (18.9)	15 (23.4)	85 (22.5)	50 (34.2)	11 (19.0)
Asian	17 (3.5)	2 (3.1)	13 (3.4)	2 (1.4)	2 (3.4)
Other	29 (6.0)	4 (6.3)	26 (6.9)	16 (11.0)	5 (8.6)
Multiple birth,[e] n (%)	133 (27.7)	13 (20.3)	85 (22.5)	9 (6.2)	2 (3.4)
Number for whom birth weight was available[f]	459	60	353	137	49
Birth weight (kg),[f] median (range)	1.90 (0.5–5.3)	1.75 (0.5–3.9)	0.90 (0.4–5.5)	3.00 (0.5–4.1)	2.50 (0.7–4.0)
Gestational age (weeks),[f] median (range)	33.0 (23–35)	32.5 (24–35)	26.0 (22–35)	38.0 (25–42)	37.0 (24–43)
<28, n/N[f] (%)	28/481 (5.8)	7/64 (10.9)	254/377 (67.4)	6/141 (4.3)	3/57 (5.3)
28– <32, n/N[f] (%)	100/481 (20.8)	22/64 (34.4)	80/377 (21.2)	11/141 (7.8)	6/57 (10.5)
32– <36, n/N[f] (%)	353/481 (73.4)	35/64 (54.7)	43/377 (11.4)	18/141 (12.8)	12/57 (21.1)
36, n/N[f] (%)	0/481 (0.0)	0/64 (0.0)	0/377 (0.0)	106/141 (75.2)	36/57 (63.2)
Palivizumab use,[g] n/N (%)	116/481 (24.1)	11/63 (17.5)	215/377 (57.0)	59/146 (40.4)	23/58 (39.7)
Palivizumab use by gestational age (weeks),[f] n/N (%)					
<28	18/28 (64.3)	3/7 (42.9)	148/254 (58.3)	3/6 (50.0)	3/3 (100.0)
28– <32	43/100 (43.0)	5/21 (23.8)	49/80 (61.3)	8/11 (72.7)	1/6 (16.7)
32– <36	55/353 (15.6)	3/35 (8.6)	18/43 (41.9)	11/18 (61.1)	7/12 (58.3)
36	0/0 (NA)	0/0 (NA)	0/0 (NA)	36/106 (34.0)	12/36 (33.3)
Prior NICU admission[f], n/N (%)	310/388 (79.9)	42/51 (82.4)	299/300 (99.7)	73/114 (64.0)	39/45 (86.7)
Prior NICU stay (days),[f] median (range)	18.0 (1–136)	28.0 (2–180)	90.0 (4–320)	33.0 (3–195)	43.5 (1–240)
Breast-fed,[f] n/N (%)	91/390 (23.3)	1/51 (2.0)	21/301 (7.0)	22/115 (19.1)	1/47 (2.1)

Abbreviations: CLD, chronic lung disease; CHD, congenital heart disease; NA, not applicable; NICU, neonatal intensive care unit.

[a]Premature defined as <36 weeks' gestation.

[b]Subjects with or without CLD.

[c]Subjects ≤12 months of age without CHD or CLD.

[d]Subjects ≤24 months of age without CHD.

[e]Being 1 of 2 or more fetuses carried to birth in a single pregnancy.

[f]Total number (N) and percentages are of those with data available. Some data were only collected in subjects enrolled after October 1, 2003.

[g]Within 1 month before enrollment.

Table 2. Prevalence of Human Metapneumovirus and Other Respiratory Viruses in Evaluable Cohorts[a]

	HMPV	RSV	PIV			INF A/B	Virus Not Identified[b]
			1	2	3		
Northern hemisphere[c]	95/948 (10%)[d]	434/961 (45%)	10/962 (1%)	1/963 (0%)	30/961 (3%)[e]	35/960 (4%)	361/936 (39%)
Southern hemisphere[c]	4/156 (3%)[d]	64/155 (41%)	1/156 (1%)	0/156 (0%)	13/156 (8%)[e]	7/155 (5%)	66/152 (43%)
Global total	99/1104 (9%)	498/1116 (45%)	11/1118 (1%)	1/1119 (0%)	43/1117 (4%)	42/1115 (4%)	427/1088 (39%)

Abbreviations: HMPV, human metapneumovirus; INF, influenza; PCR, polymerase chain reaction; PIV, parainfluenza; RSV, respiratory syncytial virus.

[a]The total numbers (denominators) of subjects evaluated differ among groups because PCR results were not always available for every pathogen.

[b]Includes all subjects in which none of the viruses tested were identified.

[c]See Methods regarding the seasons of specimen collection.

[d]10% versus 3%, $P = .001$ (Fisher's exact test).

[e]3% versus 8%, $P = .005$ (Fisher's exact test).

infection alone (data not shown). Other coinfections involving HMPV were not identified.

Demographic and clinical characteristics of HMPV-positive subjects (without coinfection) are shown in Tables 3 and 4 and the Supplemental Table. The median age of HMPV-positive subjects was 8.2 months, 60.4% were male, 53.8% were white, and 94.5% were born at <36 weeks gestational age with 52.7% at <28 weeks. Subjects with HMPV and available data presented with cough (95.6%), requirement of supplemental oxygen (82.0%), nasal congestion (74.2%), temperature >38°C (60.0%), wheezing (55.3%), tachypnea (54.8%), and apnea (8.6%) (see Supplemental Table). The median duration of hospitalization was 6.0 days (range, 1–>28 days); 23.0% (95% CI, 15%–33%) of subjects were admitted to intensive care unit; and 11% (95% CI, 5%–19%) of subjects required mechanical ventilation. The median durations of intensive care and mechanical ventilation were 4.0 days (range, 1–23 days) and 6.5 days (range, 1–24 days), respectively (see Supplemental Table).

Comparing characteristics of subjects hospitalized with HMPV and RSV (Table 3), the median age of children infected with HMPV was higher than in those with RSV (8.2 vs 4.0 months; $P < .001$), whereas the median gestational age was higher in subjects infected with RSV than in those with HMPV (33.0 vs 27.0 weeks, respectively; $P < .001$) (Table 3). Children with HMPV were more likely to have received palivizumab in the month before admission ($P < .001$), and subjects infected with HMPV were febrile at admission more frequently (60.0% vs 35.8%; $P < .001$) and had a longer duration of wheezing than the subjects infected with RSV (median, 3.0 vs 2.0 days; $P = .005$). All other clinical features and outcomes were similar (see Supplemental Table).

Table 4 compares the risk factors in children hospitalized with HMPV and RSV. Prematurity with age ≤6 months (without CLD or CHD) was more common in subjects who were RSV-positive than in subjects who were HMPV-positive ($P < .001$). In contrast, prematurity with CLD was more common in subjects who were HMPV-positive ($P < .001$). Few subjects with CHD had HMPV. More patients with HMPV had a household contact aged <6 years or 6–12 years ($P = .021$ and $P = .009$, respectively; data not shown). The same was found for RSV LRTI for household contacts aged <6 years ($P = .021$; data not shown). When risk factors were compared between subjects with HMPV and RSV, those with HMPV tended to have more children 6–12 years of age in the household (39.6%) than did those with RSV (25.5%; $P = .007$). A family history of asthma, allergies, or smokers was similar in both groups (data not shown). Daycare attendance was similar between those with HMPV (7.3%) and those with RSV (10.2%; $P = .541$).

Multivariate analysis (ie, logistic regression), which compared children hospitalized with HMPV to all others subjects, revealed an increased risk of HMPV hospitalization for older chronological age in months (odds ratio [OR], 1.048; 95% CI, 1.007–1.091). The risk of HMPV decreased at older gestational ages in weeks (OR, 0.897; 95% CI, 0.849–0.949). In addition, it revealed an increased risk of HMPV hospitalization for higher numbers of children in the household 6–12 years of age (OR, 1.411; 95% CI, 1.118–1.781) but a decreased risk for HMPV hospitalization for higher numbers of children in the

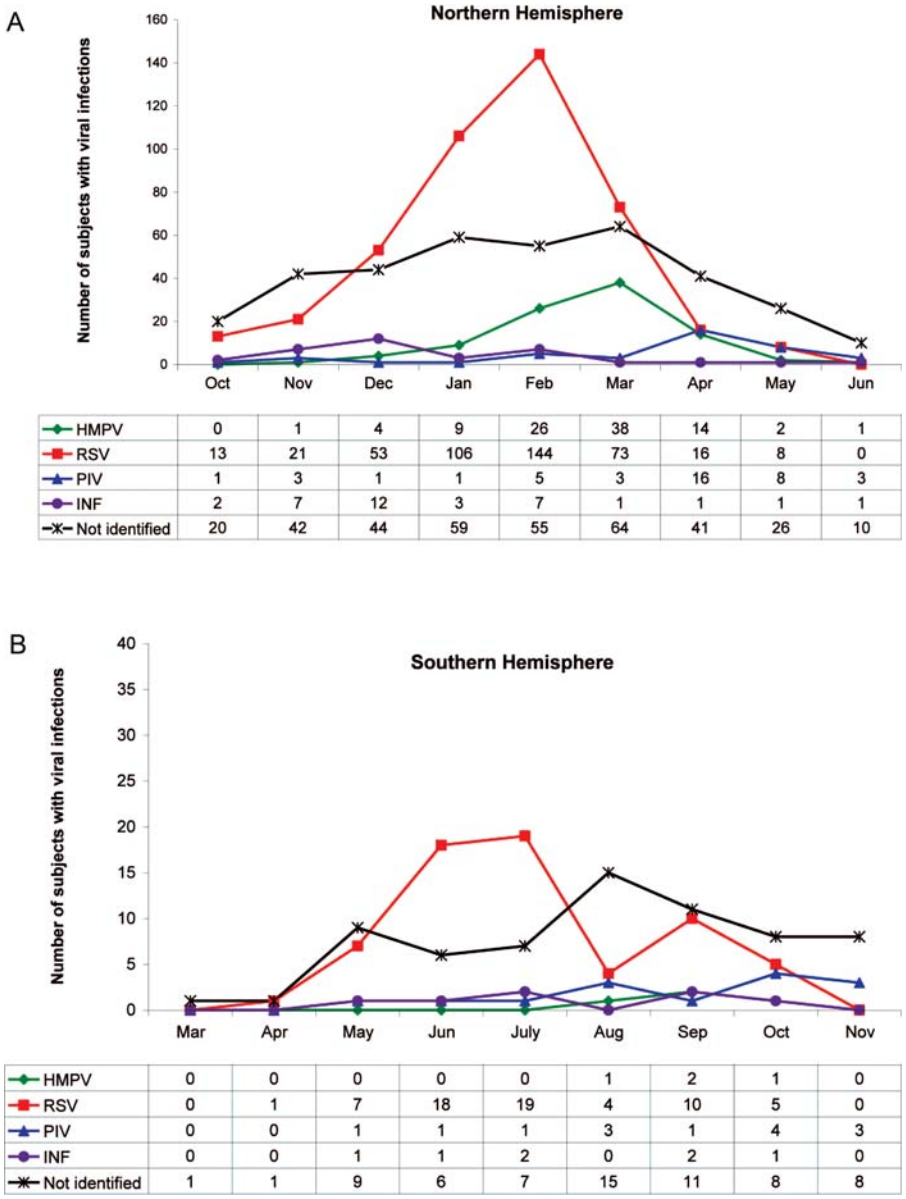

Figure 2. Number of subjects with respiratory syncytial virus and human metapneumovirus infections by month. Surveillance was conducted between October 1 and June 30 for northern hemisphere sites (*A*) and between March 1 and November 30 for southern hemisphere sites (*B*). Not every subject had results available for all of the viruses tested (see Figure 1). See Methods for additional information. Abbreviations: HMPV, human metapneumovirus; INF, influenza; PIV, parainfluenza; RSV, respiratory syncytial virus.

household >12–18 years of age (OR, 0.324; 95% CI, 0.139–0.754).

DISCUSSION

A previous surveillance study found that the annual hospitalization rate for HMPV was 1.2 of 1000 children <5 years of age, a rate similar to influenza and PIV-3 but almost one third of the RSV burden [17]. Prospective studies regarding the epidemiology of HMPV are limited and have been for short periods (eg, 2 years) in limited geographic areas [17, 18]. In our large prospective, multinational, multiyear observational study of HMPV in high-risk children hospitalized with LRTI, we identified HMPV in 9% of children (comparable to other reported rates of 4%–17.5% in children [8, 9, 17, 23, 24]).

Consistent with previous reports [4, 5, 8, 9, 17], we found that the peak HMPV season occurred slightly later in the season and in older children (median age, 8.2 months) than did RSV (median age, 4.0 months) [4, 7, 10]. In contrast to some studies [13–15], we

Table 3. Demographics and Baseline Characteristics of Subjects Hospitalized With Human Metapneumovirus, Respiratory Syncytial Virus, and No Virus Identified[a]

	HMPV-Positive (n = 91)	RSV-Positive (n = 490)	*P* Value[b]	Virus Not Identified[c] (n = 427)
Age (months), median (range)	8.20 (0.9–24.0)	3.95 (0.4–23.0)	<.001	5.80 (0.5–23.8)
Male, n (%)	55 (60.4)	282 (57.6)	.645	255 (59.7)
Race/ethnicity, n (%)				
White/non-Hispanic	49 (53.8)	268 (54.7)	.413	197 (46.1)
Black	19 (20.9)	80 (16.3)		80 (18.7)
Hispanic	19 (20.9)	92 (18.8)		103 (24.1)
Asian	1 (1.1)	16 (3.3)		14 (3.3)
Other	3 (3.3)	34 (6.9)		33 (7.7)
Multiple birth,[d] n (%)	14 (15.4)	116 (23.7)	.100	87 (20.4)
Gestational age (weeks),[e,f] median (range)	27.0 (23.0–40.0)	33.0 (23.0–42.0)	<.001	31.0 (22.0–43.0)
Gestational age (weeks),[e,f] n (%)				
<28	48 (52.7)	91 (18.6)	<.001	128 (30.4)
28– <32	19 (20.9)	77 (15.7)		101 (24.0)
32– <36	19 (20.9)	273 (55.7)		127 (30.2)
≥36	5 (5.5)	49 (10.0)		65 (15.4)
Palivizumab use,[g] n/N[f] (%)	53/91 (58.2)	134/489 (27.4)	<.001	194/427 (45.4)
Palivizumab use by gestational age (weeks), n/N[f] (% of each age group)[e]				
<28	35/48 (72.9)	48/91 (52.7)	.029	74/128 (57.8)
28– <32	12/19 (63.2)	30/76 (39.5)	.075	56/101 (55.4)
32– <36	5/19 (26.3)	38/273 (13.9)	.173	40/127 (31.5)
≥36	1/5 (20.0)	18/49 (36.7)	.646	23/65 (35.4)

Abbreviations: HMPV, human metapneumovirus; RSV, respiratory syncytial virus.

[a]Eight subjects with HMPV and RSV coinfection were excluded.

[b]Comparison between HMPV-positive and RSV-positive; *P* values comparing mean values obtained from Wilcoxon rank-sum test. *P* values comparing proportions obtained from Fisher's exact test; for race/ethnicity and gestational age, category *P* value comparing proportions obtained from a χ^2 test.

[c]Includes all subjects in which none of the viruses tested were identified.

[d]Being 1 of 2 or more fetuses carried to birth in a single pregnancy.

[e]Virus not identified with data available, n = 421.

[f]Total number (n) and percentages are of those with data available.

[g]Within 1 month before enrollment.

rarely found RSV coinfection and the severity of disease was not different. The small number of coinfected children precludes definite conclusions.

Our findings differed significantly from another prospective study with similar enrollment criteria from Argentina in which HMPV was found in only 2% of respiratory illnesses in 194 high-risk infants and young children followed until 2 years of age or study completion [18]. During the winter months, HMPV accounted for 5% of respiratory illnesses, which is similar to the 3% observed in our southern hemisphere sites (Australia). Most of the 567 respiratory illnesses in those children occurred in the outpatient setting (hospitalization occurred in only 16%), and a virus was identified in a small proportion of illnesses (15%) [18]. Thus, our data complement these primarily outpatient data by describing the pathogens isolated from high-risk children who required hospitalization for LRTI.

We found a significant difference in the burden of HMPV and PIV-3 between the northern and southern hemispheres. Overall, 3% of subjects in the southern hemisphere had HMPV and 8% had PIV-3, corresponding to the results from Victoria, Australia of a 3.1% prevalence of HMPV and 8.6% of PIV in samples obtained from hospitalized children 0–4 years of age during 2002–2003 [25]. In contrast, a study from Queensland, Australia found HMPV in 7.1% and PIV-3 in 2.3% of over 10,000 respiratory samples collected from adults and children in 2001–2004 [26].

Table 4. Underlying Risk Factors in Those Hospitalized With Human Metapneumovirus, Respiratory Syncytial Virus, and No Virus Identified[a]

Risk Factor	HMPV-Positive (n = 91) n (%) [95% CI]	RSV-Positive (n = 490) n (%) [95% CI]	*P* Value[b]	Virus Not Identified[c] (n = 427) n (%) [95% CI]
Prematurity only	30 (33.0) [23.5, 43.6]	312 (63.7) [59.2, 67.9]	<.001	154 (36.1)
Premature and age ≤6 months	25 (27.5) [18.6, 37.8]	275 (56.1) [51.6, 60.6]	<.001	139 (32.6)
Premature and age >6–12 months	5 (5.5) [1.8, 12.4]	37 (7.6) [5.4, 10.3]	.659	15 (3.5)
Premature with CLD	54 (59.3) [48.5, 69.5]	107 (21.8) [18.3, 25.8]	<.001	178 (41.7)
CLD and age ≤12 months	29 (31.9) [22.5, 42.5]	75 (15.3) [12.2, 18.8]	<.001	123 (28.8)
CLD and age >12–24 months	25 (27.5) [18.6, 37.8]	32 (6.5) [4.5, 9.1]	<.001	55 (12.9)
CHD and age ≤12 months	6 (6.6) [2.5, 13.8]	50 (10.2) [7.7, 13.2]	.338	66 (15.5)
Cyanotic	2 (2.2) [0.3, 7.7]	9 (1.8) [0.8, 3.5]	.685	12 (2.8)
Acyanotic	4 (4.4) [1.2, 10.9]	41 (8.4) [6.1, 11.2]	.283	54 (12.6)
GA <36 weeks without CLD	1 (1.1) [0.0, 6.0]	9 (1.8) [0.8, 3.5]	1.000	7 (1.6)
GA 36 weeks without CLD	5 (5.5) [1.8, 12.4]	35 (7.1) [5.0, 9.8]	.821	50 (11.7)
CHD and CLD	0 (0.0) [0.0, 4.0]	6 (1.2) [0.5, 2.6]	.597	9 (2.1)
CHD and age >12–24 months	1 (1.1) [0.0, 6.0]	21 (4.3) [2.7, 6.5]	.228	29 (6.8)
Cyanotic	0 (0.0) [0.0, 4.0]	8 (1.6) [0.7, 3.2]	.618	6 (1.4)
Acyanotic	1 (1.1) [0.0, 6.0]	13 (2.7) [1.4, 4.5]	.708	23 (5.4)

Abbreviations: CHD, congenital heart disease; CLD, chronic lung disease; GA, gestational age; HMPV, human metapneumovirus; RSV, respiratory syncytial virus.

[a]Eight subjects with HMPV and RSV coinfection were excluded.

[b]Comparison between HMPV-positive and RSV-positive; *P* values obtained from Fisher's exact test.

[c]Includes all subjects in which none of the viruses tested were identified.

Differences in medical decisions to hospitalize, differences in the ages studied, and the limited number of Australian subjects enrolled in our study may contribute to these differences.

Prematurity is a major risk factor for severe RSV and HMPV. By 3 years of age, almost 100% of children have serological evidence of RSV infection, but only 70% of children 2–5 years of age have serological evidence of HMPV infection; this increases to >90% by ages 5–9 [1, 3]. Thus, it should not be surprising that those with HMPV LRTI were generally older than those with RSV in our study and in others [4, 5, 7–10, 17]. Yet, despite the older age of those hospitalized with HMPV, they were born more prematurely than those with RSV (median, 27 vs 33 weeks; $P < .001$) and >50% of them were born at <28 weeks gestation and 74% at <32 weeks gestation. One possible explanation may be the protective effect of palivizumab against RSV infection, because 58.7% (175 of 298) of those born <28 weeks versus only 23.5% (142 of 603) of those born ≥32 weeks received palivizumab within 1 month of enrollment. In contrast, 55.7% of those with RSV were born between 32 and <36 weeks gestation, suggesting more palivizumab usage in those of lower gestational age than those 32–<36 weeks gestation (due to more restricted recommendations, eg, need of at least 2 risk factors). Although the study was not designed to evaluate the 32–<36 week cohort, potentially even more infants in this substantial subset may be at risk for RSV infections with the current American Academy of Pediatrics' Committee on Infectious Disease recommendations limiting palivizumab use in this population [27]. Thus, the importance of developing a safe and effective RSV vaccine cannot be overemphasized. In addition, because those born at <28 weeks gestation account for >50% of HMPV infections, and the additional 21% born at 28–<32 weeks are probably not protected by maternal antibodies, these subgroups should be targeted for prophylactic intervention with a safe and effective vaccine or monoclonal antibody product for HMPV, if developed.

Notably, almost 60% of HMPV-infected subjects had underlying CLD. This increased risk of HMPV infection in subjects with CLD continued through the first 2 years of life. Thus, 25 of 54 (46%) of children with CLD hospitalized with HMPV were older than a year, compared with 87 of 285 (31%) with other causes of respiratory infections. More subjects with HMPV had fever and suffered a longer duration of wheezing before hospital admission than those infected with RSV. Although others reported that HMPV

infection is less severe than RSV [9], we found no difference.

Studies have shown that RSV infection has been consistently associated with the number of siblings 2 years but <12 years and with the number of children in the household that attend school [28–30]. Interestingly, multivariate analysis of our data showed that compared with other respiratory infections, HMPV hospitalization was associated with increasing numbers of children 6–12 years of age in the household but not with increasing numbers of younger or older children. It seems that these grade school age siblings either are more commonly infected with or transmit HMPV more efficiently than their high-risk siblings. Our study supports an older age of hospitalization of those with HMPV LRTI, which is further confirmed with the higher proportion of those age >12 months with CLD who are hospitalized with HMPV. Thus, although our study was limited to children under 2 years of age, the demographic data and the multivariate analysis suggest that HMPV may play an expanded role in causing severe LRTI hospitalizations in older high-risk children.

Limitations of our study include the fact that some respiratory pathogens, whose significance has recently been appreciated, were not tested (eg, coronaviruses, bocaviruses, rhinoviruses, enteroviruses), which may explain why we identified an etiologic agent in 60% of the subjects. Including these viruses in a diagnostic panel has resulted in 93% identification [31]. It is also possible that some of the subjects had a noninfectious acute worsening of their underlying CHD or CLD. Detection of HMPV genetic material in a respiratory sample does not prove that it caused the LRTI because viral pathogens, including HMPV, can increase the risk of subsequent pneumococcal or other bacterial pneumonia [32]. Finally, the study population does not allow us to extrapolate our findings to the developing world or draw additional conclusions about differences between the hemispheres.

In conclusion, we found HMPV to be the second most common etiologic agent in the northern hemisphere in infants and young children hospitalized with LRTI that have coexistent prematurity, CLD, or hemodynamically significant CHD. Compared with children who are not infected with HMPV (including RSV), HMPV-infected high-risk subjects are older and were born more prematurely. Efforts to develop immunoprophylactic or therapeutic strategies should be intensified for high-risk infants and young children because HMPV is the etiologic agent of a substantial proportion of LRTIs requiring hospitalization in these children.

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References

1. van den Hoogen BG, de Jong JC, Groen J, et al A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med **2001**; 7:719–24.
2. Xiao NG, Xie ZP, Zhang B, et al Prevalence and clinical and molecular characterization of human metapneumovirus in children with acute respiratory infection in China. Pediatr Infect Dis J **2010**; 29:131–4.
3. Don M, Korppi M, Valent F, et al Human metapneumovirus pneumonia in children: results of an Italian study and mini-review. Scand J Infect Dis **2008**; 40: 821–6.
4. Calvo C, Pozo F, Garcia-Garcia ML, et al Detection of new respiratory viruses in hospitalized infants with bronchiolitis: a three-year prospective study. Acta Paediatr **2010**; 99:883–7.
5. Williams JV, Harris PA, Tollefson SJ, et al Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. N Engl J Med **2004**; 350:443–50.
6. Hamelin ME, Abed Y, Boivin G. Human metapneumovirus: a new player among respiratory viruses. Clin Infect Dis **2004**; 38:983–90.
7. van den Hoogen BG, van Doornum GJ, Fockens JC, et al Prevalence and clinical symptoms of human metapneumovirus infection in hospitalized patients. J Infect Dis **2003**; 188:1571–7.
8. Esper F, Martinello RA, Boucher D, et al A 1-year experience with human metapneumovirus in children aged <5 years. J Infect Dis **2004**; 189:1388–96.
9. Boivin G, De Serres G, Cote S, et al Human metapneumovirus infections in hospitalized children. Emerg Infect Dis **2003**; 9:634–40.
10. Mullins JA, Erdman DD, Weinberg GA, et al Human metapneumovirus infection among children hospitalized with acute respiratory illness. Emerg Infect Dis **2004**; 10:700–5.
11. Falsey AR. Human metapneumovirus infection in adults. Pediatr Infect Dis J **2008**; 27:S80–3.
12. Walsh EE, Peterson DR, Falsey AR. Human metapneumovirus infections in adults: another piece of the puzzle. Arch Intern Med **2008**; 168:2489–96.
13. Greensill J, McNamara PS, Dove W, et al Human metapneumovirus in severe respiratory syncytial virus bronchiolitis. Emerg Infect Dis **2003**; 9:372–5.
14. Semple MG, Cowell A, Dove W, et al Dual infection of infants by human metapneumovirus and human respiratory syncytial virus is strongly associated with severe bronchiolitis. J Infect Dis **2005**; 191:382–6.
15. Konig B, Konig W, Arnold R, et al Prospective study of human metapneumovirus infection in children less than 3 years of age. J Clin Microbiol **2004**; 42:4632–5.
16. Zhang SX, Tellier R, Zafar R, et al Comparison of human metapneumovirus infection with respiratory syncytial virus infection in children. Pediatr Infect Dis J **2009**; 28:1022–4.
17. Williams JV, Edwards KM, Weinberg GA, et al Population-based incidence of human metapneumovirus infection among hospitalized children. J Infect Dis **2010**; 201:1890–8.
18. Klein MI, Coviello S, Bauer G, et al The impact of infection with human metapneumovirus and other respiratory viruses in young infants and children at high risk for severe pulmonary disease. J Infect Dis **2006**; 193: 1544–51.
19. Maertzdorf J, Wang CK, Brown JB, et al Real-time reverse transcriptase PCR assay for detection of human metapneumoviruses from all known genetic lineages. J Clin Microbiol **2004**; 42:981–6.
20. Fan J, Henrickson KJ, Savatski LL. Rapid simultaneous diagnosis of infections with respiratory syncytial viruses A and B, influenza viruses A and B, and human parainfluenza virus types 1, 2, and 3 by multiplex quantitative reverse transcription-polymerase chain reaction-enzyme hybridization assay (Hexaplex). Clin Infect Dis **1998**; 26:1397–402.
21. Hu A, Colella M, Tam JS, et al Simultaneous detection, subgrouping, and quantitation of respiratory syncytial virus A and B by real-time PCR. J Clin Microbiol **2003**; 41:149–54.
22. Lehmann EL, Romano JP. Generalizations of the familywise error rate. Annals of Statistics **2005**; 33: 1138–54.
23. Viazov S, Ratjen F, Scheidhauer R, et al High prevalence of human metapneumovirus infection in young children and genetic heterogeneity of the viral isolates. J Clin Microbiol **2003**; 41:3043–5.
24. Esper F, Boucher D, Weibel C, et al Human metapneumovirus infection in the United States: clinical manifestations associated with a newly emerging respiratory infection in children. Pediatrics **2003**; 111: 1407–10.
25. Druce J, Tran T, Kelly H, et al Laboratory diagnosis and surveillance of human respiratory viruses by PCR in Victoria, Australia, 2002–2003. J Med Virol **2005**; 75:122–9.
26. Sloots TP, Mackay IM, Bialasiewicz S, et al Human metapneumovirus, Australia, 2001–2004. Emerg Infect Dis **2006**; 12:1263–6.
27. Krilov LR, Weiner LB, Yogev R, et al The 2009 COID recommendations for RSV prophylaxis: issues of efficacy, cost, and evidence-based medicine. Pediatrics **2009**; 124:1682–4.
28. Simoes EA. Environmental and demographic risk factors for respiratory syncytial virus lower respiratory tract disease. J Pediatr **2003**; 143:S118–26.
29. Simoes EA, Carbonell-Estrany X, Fullarton JR, et al A predictive model for respiratory syncytial virus (RSV)

hospitalisation of premature infants born at 33–35 weeks of gestational age, based on data from the Spanish FLIP Study. Respir Res **2008**; 9:78.
30. Carbonell-Estrany X, Simoes EA, Fullarton JR, et al Validation of a model to predict hospitalization due to RSV of infants born at 33–35 weeks' gestation. J Perinat Med **2010**; 38:411–7.
31. Suryadevara M, Cummings E, Bonville CA, et al Viral etiology of acute febrile respiratory illnesses in hospitalized children younger than 24 months. Clin Pediatr (Phila) **2011**; 50:513–7.
32. Ampofo K, Bender J, Sheng X, et al Seasonal invasive pneumococcal disease in children: role of preceding respiratory viral infection. Pediatrics **2008**; 122:229–37.