

# A community study of clinical traits and risk factors for human metapneumovirus and respiratory syncytial virus infection during the first year of life

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**Abstract** Human metapneumovirus (hMPV) and respiratory syncytial virus (RSV) are important respiratory pathogens with similar symptomatology. The aim of this prospective birth cohort study was to identify risk factors for an hMPV or RSV infection during the first year of life in unselected healthy children. We followed 217 children from birth to 1 year of age. Nasal swabs and symptom diaries were collected monthly. Anti-hMPV and anti-RSV IgG antibodies by age 1 year were detected by ELISA, and nasal swabs were analysed for hMPV and RSV by RT-PCR. Logistic regression was used for risk factor analysis. Anti-hMPV IgG was found in 38 children (17.5%), and anti-RSV IgG in 172 children (79%). Risk factors for being anti-hMPV IgG-positive were: (1) being born in the spring (OR=2.36; 95% CI:1.06–5.27), and (2) having older

siblings (OR=3.82; 95% CI:1.75–8.34). Risk factors for being anti-RSV IgG-positive were: (1) gestational age <38 weeks (OR=3.39; 95% CI:1.42–8.05), (2) increasing paternal age (OR=1.85 per 5 yrs; 95% CI:1.28–2.68), and (3) wall-to-wall carpeting (OR=3.15; 95% CI:1.29–7.68). Being born in the spring was associated with decreased odds of being anti-RSV IgG-positive (OR=0.27, 95% CI:0.09–0.85). Risk factors for RSV hospitalisation ( $n=11$ ) were: (1) older siblings (OR=4.49; 95% CI: 1.08–18.73) and (2) smoking in the household (OR=5.06; 95% CI: 1.36–18.76). Exclusive breastfeeding for the first 14 days of life protected against hospitalisation (OR=0.21; 95% CI:0.06–0.79). In conclusion, this study identifies risk factors for mild and asymptomatic hMPV infections in infancy.

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## Abbreviations

hMPV	human metapneumovirus
RSV	respiratory syncytial virus
OR	odds ratio
PBS	phosphate-buffered saline
NPA	nasopharyngeal aspirate
RT-PCR	reverse transcription-polymerase chain reaction
ELISA	enzyme-linked immunosorbent assay
RT	room temperature
M-PBS	PBS with 2% skim-milk powder
HRP	horseradish peroxidase
TMB	tetramethyl benzidine
OD	optical density
SD	standard deviation
PDV	Phocine Distemper Virus

Ct	threshold cycle
CV	coefficient of variation
CI	confidence interval

## Introduction

Since its discovery by Dutch researchers in 2001, human metapneumovirus (hMPV) has been found in all age groups worldwide [15]. A number of studies have compared the clinical symptoms associated with hMPV infection in hospitalised children with those associated with respiratory syncytial virus (RSV) infections [1, 3, 12, 27, 28, 36, 37, 39, 42]. Although hMPV infections in many studies tend to be slightly milder than RSV infections, a statistically significant difference was not observed. Few studies have reported longer hospital stays for hMPV-infected children than for RSV-infected children [25, 28].

Most studies have shown that around 20% of children acquire hMPV infection during their first year of life [22, 35, 43], while approximately 70% acquire RSV infection [13]. One to 3 percent of RSV-infected children get hospitalised, and several studies have identified a number of risk factors for severe RSV infection [2, 5, 20, 26, 32]. A recent study suggests that risk factors for hMPV hospitalisation appear to be similar to risk factors for RSV hospitalisation [30]. However, risk factors leading to hospitalisation in high-risk groups are not necessarily identical to risk factors for infection in the general population. Little is known about factors protecting against hospitalisation for hMPV infection. As both hMPV and RSV infection have been associated with subsequent wheezing [11, 19, 21, 33], it is important to investigate risk factors for early infection in order to prevent or postpone infection in predisposed individuals.

In the present study we followed a cohort of healthy infants from birth to 1 year of age. The aim of the study was to identify risk factors for an early hMPV or RSV infection in otherwise healthy children in the community. This information will help recognize children at risk of an early infection who might benefit from preventive intervention strategies.

## Materials and methods

### Study population

Children were enrolled from the post-natal ward at Copenhagen University Hospital, Hvidovre, Denmark. To ensure that children were sampled equally throughout the year, we aimed at including a predetermined number of 20

children each month during a 12-month period from May 2004 to May 2005. The inclusion criteria for participation in the study were: (1) infants free of obvious health problems and (2) for practical purposes living within a radius of 11 km from the hospital. Exclusion criteria were: (1) infants whose parents did not understand or speak Danish or English, (2) infants whose mothers had a serious psychiatric disorder, (3) infants with congenital diseases, and (4) if change of address to outside the area of Hvidovre Hospital was planned within 12 months of enrolment. Written informed consent was obtained from the parents of the infant after they had been informed about the study and before performance of any study procedure. Study approval was obtained from the Ethics Committee of Frederiksberg, Copenhagen, Denmark.

### Sample size calculation

We assumed that 75% and 25% of the children became infected with RSV and hMPV, respectively, during the first year of life. With a chosen sample size of 200 subjects, it would be possible to detect risk factors for RSV or hMPV infection during the first year of life, with relative risks of 1.3 and 2.1 respectively. Differences in symptoms related to RSV and hMPV infection could be detected with an odds ratio (OR) of 2.8, under the assumption of 5% significance level and 80% strength. Power calculations were made in EpiInfo 2000.

### Clinical data

Parents were provided monthly with a health diary displaying 12 different symptoms and clinical signs: nasal discharge, cough, fever/feels hot, conjunctivitis, fast breathing, wheezing, hoarseness, skin rash, reduced appetite, vomiting, diarrhoea (>3 watery stools/day), general malaise and space to provide information about doctor's visits, hospital admissions and medicine. Parents were encouraged to complete the diary every day. Children were monitored through monthly home visits by a paediatrician (MLL), a study nurse or a study physician. At every home visit, children had a nasal swab taken, the symptom diary from the preceding month was collected and any queries were rectified.

At the first home visit, the parents were interviewed about household contacts, parents' education and employment, ethnicity, birth weight of the child, breastfeeding, dispositions (hay fever, asthma and atopic dermatitis) and exposures (smoking in homes, smoking during pregnancy, pets, moisture, carpets, drying clothes inside). Questions concerning factors that could change over time were repeated every second month. Socioeconomic status was classified according to the Danish social classification

system, choosing the social group which comes first in the order 1 to 5 in families where the mother and father were classified in two different categories [16].

#### Definition of illness episode

An hMPV and RSV illness episode included the day of the hMPV or RSV-positive test, and was defined as time from first day with symptoms to last day with symptoms without symptom-free days in the period. All symptoms occurring during the episode were considered associated with the hMPV or RSV infection. If symptoms persisted for more than 1 month, and an hMPV or RSV-negative nasal swab specimen was available from the month before or the month after the positive test, the episode was considered terminated on the dates of collecting the negative samples.

#### Specimen collection

Nasal swabs were obtained from both nostrils in children younger than 6 months of age, and from one nostril in older children. Samples were collected from a depth of 2 to 3 cm by using a sterile thin cotton swab on an aluminium shaft that was then placed into a vial containing 1 ml of viral transport medium (bovine serum albumin and antibiotics in phosphate-buffered saline [PBS]). Approximately 80% of specimens were frozen at  $-80^{\circ}\text{C}$  within 7 hours. On some occasions the parents were instructed in sampling technique, and sent the samples by mail to the laboratory. On arrival, these samples were initially stored at  $+4^{\circ}\text{C}$  and frozen at  $-80^{\circ}\text{C}$  within 1 day of collection. We evaluated the sensitivity of the nasal swabs compared with nasopharyngeal aspirates (NPA) in 20 children hospitalised with RSV infection. We found RSV RNA in all samples by reverse transcription-polymerase chain reaction (RT-PCR), although most nasal swabs contained lower amounts of viral RNA.

Blood samples were collected from the mothers as soon as their child was enrolled in the study, and from children at age 5 days and 12 months. Samples were centrifuged at 3000 rpm for 10 min, and divided into cells and plasma.

#### RSV enzyme-linked immunosorbent assay (ELISA)

ELISA was used to determine anti-RSV IgG in plasma from mothers shortly after delivery and from 1-year-old children. Ninety-six-microwell, high-binding, half-area ELISA plates (Corning) were coated with 50  $\mu\text{l}$  of 2  $\mu\text{g}/\text{ml}$  purified RSV antigen (8RSV79, HyTest, Finland) in 50 mM carbonate buffer (pH 9.6), and incubated overnight at  $+4^{\circ}\text{C}$ . The plates were washed two times with PBS containing 0.05% Tween-20 (Merck KGaA), and blocked for 75 min at room temperature (RT) with PBS containing 2% skim milk powder (M-PBS). Plates were washed, and serial dilutions

of the positive control (3.86 mg/ml) were added, together with plasma samples diluted 1:1000 (maternal samples) or 1:100 (children's samples) in M-PBS and a negative control (M-PBS). Samples were measured in duplicate. Plates were incubated for 1 h at RT. Next, plates were washed four times and incubated for 1 h at RT with horseradish peroxidase (HRP)-conjugated goat-anti-human IgG (Star 106P, AbD Serotec, UK) diluted 1:3000 in M-PBS. The plates were washed again four times, and antibodies were detected by adding 50  $\mu\text{l}$  of 3,3', 5,5'- tetramethyl benzidine (TMB) substrate (AdB Serotec, UK). After 10 min, the reaction was stopped using 50  $\mu\text{l}$  of 1 M  $\text{H}_2\text{SO}_4$ . The extinction was measured at 450 nm.

Logarithmic standard curves were generated for the RSV ELISA with a coefficient of regression of 0.98 and an interassay variation ranging from 0 to 25%, with a mean coefficient of variation (CV) of 10%. Intraassay repeatability was measured on duplicate samples with a mean CV of 6.3%.

#### hMPV ELISA

hMPV antigen was prepared by sonicating infected LLC-MK2 cells in 50 mM carbonate buffer (pH 9.6). Sonicated cell pellet from the same cell line negative for hMPV was prepared the same way and used as negative control antigen. As positive and negative controls, we used sera from two anti-hMPV IgG-positive persons and two anti-hMPV IgG-negative persons respectively. ELISA plates were coated in every second well with 50  $\mu\text{l}$  of hMPV antigen and 50  $\mu\text{l}$  of negative control antigen respectively, diluted 1:200 in 50 mM carbonate buffer (pH 9.6) and incubated overnight at  $+4^{\circ}\text{C}$ . The plates were washed three times with PBS containing 0.25% Tween-20. Plasma samples and negative controls, diluted 1:100 in M-PBS, were added in addition with serial dilutions of the positive control, and plates were incubated for 1 h at RT. Next, plates were washed four times and incubated for 1 h at RT with HRP-conjugated goat-anti-human IgG. The plates were washed again four times, and the presence of hMPV antibodies were detected by adding 50  $\mu\text{l}$  of TMB substrate and then 50  $\mu\text{l}$  of 1 M  $\text{H}_2\text{SO}_4$  to stop the reaction. The extinction was measured at 450 nm with a reference filter at 630 nm. Net optical density (OD) values were calculated as OD values for the antigen minus OD values for the control antigen. hMPV inter- and intraassay variation were 7.4% and 3.8% respectively, based on OD values.

The cut-off value of each hMPV and RSV assay was defined by adding 2 standard deviations (SD) to the mean OD of all negative sera.

#### Real-time RT-PCR analysis of hMPV and RSV

Nucleic acid purification was carried out using the Total Nucleic Acid Isolation Kit (Roche Applied Science,

Mannheim, Germany) in combination with the Total NA Variable Elution Volume protocol on a MagNA Pure LC Instrument (Roche Applied Science) according to the instructions of the manufacturer. We used 190  $\mu$ l of material for purification, which was eluted into a final volume of 100  $\mu$ l. Prior to purification, 10  $\mu$ l of phocine distemper virus (PDV) were added to the sample as an internal control, in a concentration titrated to yield a threshold cycle (Ct) value of approximately 32 upon real-time RT-PCR analysis. Positive and negative controls were included in purification and PCR procedures. Real-time RT-PCR analyses of hMPV, RSV-A, RSV-B and PDV from each sample were carried out in four separate reactions using the TaqMan EZ RT-PCR Kit (Applied Biosystems, Foster City, CA, USA). The sequences of hMPV primers and probes were as previously published [24]. The PCR analysis was performed on a 7500 Real-Time PCR System (Applied Biosystems). To confirm a successful purification and amplification and to assess any presence of PCR inhibitors in the sample, the actual Ct value of the PDV reaction for each sample was compared with the expected Ct value. Comparisons of PDV Ct values were made using the MedLab QC freeware programme.

All hMPV and RSV-positive samples were tested for co-infection with rhinoviruses, adenovirus, influenzaviruses A and B, parainfluenzaviruses 1–3, coronaviruses OC43, 229E, and NL63, by real-time PCR using a protocol, primer/probe mixes, and positive controls obtained from Bert Niesters, Erasmus Medical Centre, Rotterdam, The Netherlands. Analyses for coronavirus HKU1 and human bocavirus were performed by an in-house PCR at the Department of Microbiology, Hvidovre Hospital, Copenhagen, Denmark, by using the same system as described above.

#### Statistical methods

For comparisons between symptoms of the hMPV and RSV-positive children, Fisher's exact test was used for discrete variables (number of children), and the non-parametric Mann-Whitney *U*-test for continuous variables (duration of symptoms and episodes).

Univariate logistic regression analysis was used to assess potential risk factors for the presence of anti-hMPV and anti-RSV IgG antibodies at age 1 year. Predictors with a *p*-value  $\leq 0.1$  were entered into a multiple logistic regression analysis. To reduce problems with co-linearity, only one or two variables from each group of covariates (social variables, smoking parameters, atopic dispositions, breastfeeding, siblings, crowding factors, and indoor environment) were included. From these common logistic regression models, backwards elimination of non-significant variables was performed using the Likelihood Ratio statistics until a *p*-level of less than 0.1 for all

variables was reached. All analyses were adjusted for sex. To test for significant effect modifications between the effect of any significant variable and that of any other significant variable on the outcome, we performed formal statistical tests for primary interaction by generating interaction terms and using them as covariates in addition to the main effects on the independent variable. A *p*-value  $< 0.05$  was considered significant. Data were analysed by using the SPSS software version 13.0 for Windows.

## Results

### Study population

Of 336 children whose parents were invited to participate in the study, 242 were accepted, of whom 217 were followed throughout 1 year. Fourteen families dropped out within the first month, and 11 children dropped out during the year. Participating infants included eight pairs of twins. One child born in week 30 was treated in the neonatal intensive care unit with nasal-continuous positive airway pressure and tube feeding. This child remained in the study as no complications emerged. Characteristics of the study population are given in Table 1.

### Anti-hMPV and anti-RSV IgG antibodies in children and mothers

To identify children who had experienced an hMPV or an RSV infection in the first year of life, we assumed that infected children developed an antibody response, which was measurable at age 12 months. Specific anti-hMPV and anti-RSV IgG was measured in plasma from the 217 children, who were followed throughout 1 year. Anti-RSV IgG antibodies were detected in 172 children (79%) (median 491 ng/ml, range 158–60,000 ng/ml) and anti-hMPV IgG was found in 38 children (17.5%) (median OD 0.44, range 0.094–1.216).

Blood samples were available from 241 mothers. All had detectable anti-RSV IgG (median 4106 ng/ml, range 1120–65,522 ng/ml) and anti-hMPV IgG (median OD 0.787, range 0.122–2.312).

### Risk factors for hMPV infection

Dates of birth were divided into four quartiles according to seasonality. In both univariate and multiple logistic regression analyses, being born in the spring (March–May) compared to the rest of the year and having older siblings were significantly associated with increased risk of detectable anti-hMPV IgG at age 12 months, while drying clothes inside during the first 6 months of life protected against infection (Table 2).



**Table 1** Demographic characteristics of 217 healthy infants followed from birth to 1 year of age, Copenhagen, 2004–2006

Sex (%)	
Boys	112 (52)
Girls	105 (48)
Gestational age (%)	
<34 wks	2 (1)
<37 wks	17 (8)
≥37 wks	198 (91)
Birth weight (%)	
<2000 g	3 (1)
<3000 g	33 (15)
≥3000 g	180 (83)
Mother's age, yrs	
Median (range)	31 (19–44)
Father's age, yrs	
Median (range)	32 (20–53)
Breastfeeding (%)	
At birth	205 (95)
At 6 months	141 (65)
At 12 months	50 (23)
Socioeconomic status <sup>a</sup> (%)	
1–2	156 (72)
3–5	56 (26)
Out of classification (students)	5 (2)
No. of siblings (%)	
0	116 (54)
1	78 (36)
2	15 (7)
≥3	8 (4)
Current smoking (at birth) (%)	
Mother	18 (8)
Father	47 (22)
Two adult smokers	14 (7)
No. of parents with asthma/hay-fever (%)	
0	116 (54)
1	88 (41)
2	10 (5)

<sup>a</sup> Socioeconomic status: 1; Long academic education (≥ 5 yrs), 2; Medium long education (3–4 yrs), 3; Short education (1–3 yrs) or independent worker, 4; skilled worker or education <1 yr, 5; Non-skilled worker/no education

### Risk factors for RSV infection and hospitalisation

Risk factors for having detectable anti-RSV IgG by age 1 year differed from those associated with hMPV infection. In univariate analysis, gestational age <38 weeks, being born in the fall (September–November), increasing paternal age, and wall-to-wall carpeting were significantly associated with RSV infection, while being born in the spring protected against infection (Table 2). In multiple analysis, gestational age <38 weeks, increasing paternal age, wall-to-wall carpeting, and being born in the spring remained significant. The effect of increasing paternal age was not explained by an increasing number of older siblings.

Maternal age was not significantly associated with RSV infection (OR=1.4 per 5 yrs; 95% CI:0.96–2.05).

As a measure of severity, we looked specifically at risk factors for RSV hospitalisation ( $n=11$ , 5.1%). In univariate analysis, smoking parameters (smoking during pregnancy, smoking in the household, number of cigarettes, number of smokers, maternal smoking, and paternal smoking) were significantly associated with increased risk of RSV hospitalisation, as were the number of children in the home and having older siblings. Full breastfeeding during the first 14 days protected against hospitalisation. In multiple analysis, breastfeeding for 14 days, having older siblings, and smoking in the household remained statistically significant (Table 2). Similar findings were observed when looking specifically at risk of hospitalisation among the anti-RSV IgG-positive children.

### Clinical symptoms

Clinical symptoms associated with hMPV or RSV infection were evaluated using nasal swabs. Due to resource constraints, and as 89% of anti-hMPV IgG-positive children were also anti-RSV IgG-positive, we chose to analyse nasal swabs from the anti-hMPV IgG-positive children only to detect the time of primary hMPV or RSV infection. Of 474 samples from these children, 155 were taken within 3 days of respiratory symptoms. These 155 samples were in addition to 31 control specimens, taken at a time when the child had been free of respiratory symptoms and fever for at least 3 weeks prior to collection to 1 week after the sample was taken, analysed for RSV A, RSV B, and hMPV by RT-PCR.

We detected ten hMPV-positive and 15 RSV-positive nasal swabs from 24 different children, corresponding to 26.3% (ten out of 38) of the hMPV infections and 8.7% (15 out of 172) of the RSV infections. RSV-positive samples were identified from children down to 26 days of age, while primary hMPV infection was only seen in children older than 6 months. One hMPV-infected infant was asymptomatic. Co-infection with other respiratory viruses, primarily rhinovirus and human bocavirus, occurred in nine cases (Table 3). Information on clinical symptoms associated with single hMPV and RSV infections was obtained from the health diaries, and is illustrated in Table 4. The groups were too small to detect any statistically significant differences in distribution and duration of symptoms.

### Discussion

The present study identifies risk factors for mild and asymptomatic hMPV infections within the first year of life in unselected healthy infants. We found that risk factors for

**Table 2** Logistic regression analyses of variables associated with hMPV infection, RSV infection, and RSV hospitalisation during the first year of life in 217 healthy infants, Copenhagen, 2004–2006

Variable	hMPV infection				RSV infection				RSV hospitalisation									
	Univariate logistic regression		Multiple logistic regression		Univariate logistic regression		Multiple logistic regression		Univariate logistic regression		Multiple logistic regression							
	OR	95% CI	<i>P</i> value	OR	95% CI	<i>P</i> value	OR	95% CI	<i>P</i> value	OR	95% CI	<i>P</i> value						
<i>n</i> (%) of infants																		
Season of birth <sup>a</sup>																		
Spring	1.90	0.90–4.01	0.09	2.36	1.06–5.27	0.036	0.29	0.11–0.80	0.016	0.27	0.09–0.85	0.025	0.63	0.13–2.99	0.56	–	–	
Summer	0.63	0.27–1.47	0.29	–	–	–	1.05	0.51–2.17	0.89	–	–	–	0.96	0.25–3.73	0.95	–	–	
Fall	0.53	0.23–1.22	0.14	–	–	–	2.07	1.05–4.06	0.035	–	–	–	0.81	0.21–3.17	0.77	–	–	
Winter	1.72	0.71–4.19	0.23	–	–	–	1.08	0.44–2.69	0.86	–	–	–	2.29	0.57–9.13	0.24	–	–	
Gestational age < 38 weeks	1.32	0.57–3.05	0.51	–	–	–	2.96	1.42–6.18	0.004	3.39	1.42–8.05	0.006	2.56	0.32–20.57	0.38	–	–	
Older siblings	3.62	1.71–7.65	0.001	3.82	1.75–8.34	0.001	0.86	0.44–1.68	0.66	–	–	–	3.79	0.98–14.73	0.054	4.49	1.08–18.73	0.04
Breastfeeding first 14 days	1.17	0.45–3.03	0.75	–	–	–	1.85	0.68–5.06	0.23	–	–	–	0.23	0.07–0.80	0.021	0.21	0.06–0.79	0.02
Maternal smoking in pregnancy	0.64	0.23–1.77	0.39	–	–	–	1.01	0.43–2.39	0.98	–	–	–	4.19	1.21–14.52	0.024	–	–	–
Smoking in household	0.55	0.21–1.39	0.21	–	–	–	0.58	0.24–1.40	0.23	–	–	–	4.21	1.23–14.45	0.022	5.06	1.36–18.76	0.015
Paternal age per extra 5 years	0.93	0.66–1.32	0.68	–	–	–	1.78	1.27–2.49	0.001	1.85	1.28–2.68	0.001	1.23	0.69–2.19	0.48	–	–	–
Wall-to-wall carpeting	0.96	0.39–2.37	0.94	–	–	–	2.43	1.14–5.15	0.021	3.15	1.29–7.68	0.012	0.42	0.05–3.34	0.41	–	–	–
Sleeps outside during the day	1.08	0.46–2.54	0.87	–	–	–	0.47	0.24–1.03	0.058	0.44	0.19–1.03	0.057	0.77	0.19–3.03	0.71	–	–	–
Drying clothes inside <sup>b</sup>	0.42	0.20–0.86	0.019	0.45	0.21–0.97	0.041	0.57	0.29–1.13	0.11	–	–	–	1.12	0.33–3.79	0.85	–	–	–

<sup>a</sup> Reference categories are all other seasons<sup>b</sup> The protective effect was present for drying clothes inside during the first 6 months of the child's life. The presented ORs are from drying clothes inside at age 1 month. Only variables significant in univariate analysis are included in the table. Adjusted for sex.

**Table 3** Distribution of other respiratory viruses identified in hMPV and RSV-positive nasal swab specimens from healthy infants, Copenhagen, 2004–2006

Viruses	hMPV-positive (n=10)	RSV-positive (n=15)
Rhinoviruses	0	3
Coronavirus OC43	0	1
Human bocavirus	1	3
Triple virus*	1	0

\* human metapneumovirus, human bocavirus, and rhinovirus

hMPV and RSV infection were different, as were risk factors for mild and severe RSV infection. The prospective design of the study and the close contact to participating families through monthly home visits resulted in low drop-out rates, and ensured a high quality of information on risk factors and symptoms during infection.

Our population consists of healthy infants, and the risk factors found are valid for normal children. Children with high-risk conditions, such as bronchopulmonary dysplasia, prematurity, cystic fibrosis, and congenital heart diseases are more prone to severe infections, and the risk factors found in this study cannot be extrapolated to such children.

The risk of attracting hMPV infection was mainly determined by the presence of older siblings, who bring the virus to the family, but also by birth in the spring. Most studies on seasonality of hMPV have found that hMPV infection peaks during late winter/early spring (January–April) [1, 12, 27, 30], although some studies report that

hMPV hospitalisation peaks in the early winter [9]. These studies also find that most children are infected after 6 months of age. Children born during spring in this cohort may mainly contract their hMPV infection at late ages (>10 months), explaining this finding. No other studies are directly comparable with ours, but studies including children with underlying pathologies have found prematurity, male gender, congenital heart disease, and gastrointestinal reflux and aspiration to be risk factors for hMPV hospitalisation [30]. In our study, gender was not a risk factor for hMPV infection.

Prematurity is a known risk factor for severe RSV infection, and even though we only included 19 babies born prematurely (weeks 30–37), we found that gestational age less than 38 weeks was associated with RSV infection, but not with hospitalisation. The effect of increasing paternal age on risk of RSV infection was new to us. The paternal age span ranged from 20–53 years, and the effect was especially noticeable among fathers older than 38 years. Young adults usually have the highest amounts of antibodies to respiratory viruses. With increasing age, antibody levels decline, making the individual more susceptible to new infections. An explanation could be that older fathers, due to low levels of RSV antibodies, become infected and transmit the virus to the child. However, we did not collect plasma samples from the fathers for antibody detection to confirm this theory. The same effect was not noticed for mother's age, probably due to a smaller age span.

Birth into the RSV season increased infection rates, which has been reported by others [5, 20, 32]. However,

**Table 4** Distribution of symptoms and clinical signs associated with single hMPV and RSV infections in healthy children, Copenhagen, 2004–2006\*

Symptom	hMPV-positive (n=8)		RSV-positive (n=8)	
	No. children (%)	Duration in days median (range)	No. children (%)	Duration in days median (range)
General malaise	2 (25)	0 (0–22)	5 (63)	3 (0–8)
Nasal discharge	8 (100)	8.5 (1–23)	7 (88)	5.5 (0–26)
Cough	4 (50)	4.5 (0–56)	6 (75)	5 (0–27)
Fever	3 (38)	0 (0–2)	5 (63)	2 (0–8)
Tachypnoea	0	0	3 (38)	0 (0–8)
Wheezing	2 (25)	0 (0–56)	3 (38)	0 (0–4)
Hoarseness	2 (25)	0 (0–24)	4 (50)	1 (0–8)
Conjunctivitis	0	0	2 (25)	0 (0–12)
Rash	1 (13)	0 (0–10)	2 (25)	0 (0–8)
Diarrhoea	2 (25)	0 (0–15)	4 (50)	1 (0–8)
Vomiting	2 (25)	0 (0–2)	3 (38)	0 (0–3)
Lost appetite	3 (38)	0 (0–11)	4 (50)	0.5 (0–3)
Duration of episode	–	18 (2–56)	–	10.5 (6–28)
Doctor's visit	3 (38)	–	6 (75)	–
Hospitalisation	0	–	3 (38)	–
Received medication	2 (25)	–	3 (38)	–

\*Differences between the RSV-positive and the hMPV-positive groups are all non-significant

this finding disappeared in multiple regression analysis, as the protective effect of being born in the spring was more pronounced. To our knowledge, the association between carpeting and RSV infection has not been described previously. It is not known whether the survival of RSV is greater on carpets than on smooth floors, but young children spend much time lying and crawling on the floor, and transmission from surfaces contaminated with RSV-infected nasal secretions is possible [14]. Maybe parents are more inclined to place their child directly on a carpet rather than on a cold floor, and maybe cleaning is easier achieved on the floor through rubbing. Factors highly associated with RSV hospitalisation were passive smoking and the presence of older siblings, while exclusive breastfeeding in the first 2 weeks of life was protective. This is in accordance with other studies of severe RSV infection [17, 26, 29].

The identification of children with a previous hMPV or RSV infection was based on IgG detection in plasma by age 1 year. In most children, maternal antibodies disappear within 6 months [4, 6, 7], and IgG detected after that age will be the result of natural infection. Some studies report that infants <6 months of age have poor serum antibody responses against RSV infection, making the use of seroepidemiological diagnosis difficult [4, 10, 41]. Other studies have found that almost all children <1 year of age generate an antibody response after their first RSV infection [31]. The seroprevalence of anti-RSV IgG in our cohort is at the higher end of what is usually reported, and seven children with proven RSV infection before 2 months of age all had detectable low levels of anti-RSV IgG by age 12 months, making the possibility of an underestimation unlikely. The seroprevalence of anti-hMPV IgG correlates well with the results of other studies [22].

The present study has certain limitations that need to be taken into account when considering the study and its contributions. The study was first and foremost designed to detect risk factors for hMPV and RSV infection in infancy. The sample size calculation for detection of differences in symptoms related to hMPV and RSV infection was based on the assumption that all hMPV and RSV infections were identified during the study period. However, we did not identify time of infection for the majority of IgG-positive children. The clinical symptoms related to hMPV and RSV infection in young children in the community in this study is therefore descriptive in nature. Shedding durations of RSV and hMPV in nasal secretions after an acute infection have been reported to be 11.5 and 5 days respectively [38]. Therefore, nasal swab specimens taken more often than once a month would be preferable to identify the time of primary infection. However, for ethical and practical reasons, this was not possible in the present study. Collecting nasal swabs at regular monthly intervals made it possible to identify a quarter of the hMPV infections. We

used this method to avoid the risk of bias and under-reporting by parents if they actively had to contact us for every respiratory episode, which has been the case in other studies [8].

Nasal swabs may not be as sensitive as NPA in detecting RSV [18, 23, 34]. However, a recent study reports that when a sensitive amplification method like RT-PCR is used for RSV analysis, the sensitivity of nasal swabs is comparable to that of NPA [40]. The swabs were chosen, as they were sensitive in our own test of RSV in hospitalised infants, and as they were much more appropriate for this epidemiological survey collecting 13 samples from each child's nose.

In conclusion, this study provides information on risk factors for mild and asymptomatic hMPV infections in unselected healthy infants, and helps identify children at risk of an early infection.

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