ORIGINAL PAPER

Clinical findings and unusual epidemiologic characteristics of human metapneumovirus infections in children in the region of Basel, Switzerland

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Abstract Human metapneumovirus (hMPV) worldwide causes respiratory tract infections with features similar to those of RSV infection. We describe features of hMPV infections in children and compare some of the characteristics with those of RSV infections. From October 2004 to February 2006, 75 patients, 34 hospitalized and 41 outpatients, were diagnosed with hMPV infections by multiplex PCR applied to nasopharyngeal specimens. While hMPV was found rarely in the early phase of the study, a significant increase occurred in the second winter of the study period. Patients with hMPV infections were older than those with RSV infection; clinical characteristics were similar as was the rate of serious disease among hospitalized patients (intensive care treatment: 18% versus 8%). In conclusion, hMPV leads to endemic and epidemic respiratory disease with features similar to those of RSV and should be considered in the differential diagnosis of upper and lower respiratory tract disease.

Keywords Human metapneumovirus (hMPV) · Respiratory tract infection (RTI) · Children

Introduction

Human metapneumovirus (hMPV) is a recently discovered paramyxovirus that is associated with acute respiratory tract infections (RTIs) in children and adults. Since its first description in 2001 [36], the virus has been further identified in patients with RTIs from all continents [1, 3,

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8, 9, 14, 15, 19, 21, 22, 27–34, 41]. Seroprevalence studies in the Netherlands [36], Israel [44] and Japan [11] have shown that by the age of 5 to 10 years, seropositivity reaches virtually 100%. Longitudinal surveys have indicated that hMPV has a seasonal distribution similar to respiratory syncytial virus (RSV) and influenza viruses [15, 18, 34, 42].

In October 2004, a reverse-transcriptase-polymerase chain-reaction (RT-PCR) assay was added to the panels of multiplex PCR in our laboratory to test nasopharyngeal aspirates from in- and outpatients with RTIs for the presence of respiratory pathogens [25]. Whereas only a small number of samples tested positive for hMPV in the first winter season after its introduction, we observed a remarkable increase of hMPV infections during the following winter season (October 2005 to February 2006). Here we present demographic and clinical features of hMPV infections in children that, to the best of our knowledge, is the first such report from Switzerland. Further, we compare some of the observed epidemiologic characteristics with those of RSV infections that were diagnosed during the same time period.

Materials and methods

Study subjects

Our microbiological laboratories provide a broad range of diagnostic services for in- and outpatients of our children's hospital. In addition, several pediatricians in the close vicinity take advantage of our services. From 11 October 2004 onwards, a RT-PCR for detection of hMPV was performed in nasopharyngeal aspirates (NP) sent to our laboratory in addition to the use of a previously established

multiplex PCR for detection of RSV, parainfluenza virus types 1 and 3, influenza A and B, and adenovirus. All assays had undergone comprehensive in-house evaluation before their introduction. NP were obtained from hospitalized patients as well as outpatients with acute RTIs according to standard clinical care in our institution and collaborating private pediatric offices. Laboratory personnel were unaware of any clinical information on the respective patients.

Clinical information (based on a standardized questionnaire) and laboratory data were abstracted from the medical records of hospitalized patients retrospectively in March 2006. For outpatients, questionnaires were sent to the pediatrician or practitioner who had collected the NP specimens. Specifically, demographic characteristics (age, gender, prematurity and underlying chronic diseases), onset of symptoms, duration of illness, clinical signs and symptoms, medication and the final clinical diagnosis were collected.

The study protocol was approved by the University of Basel Medical Faculty's ethical commission. Informed consent was obtained from the patients' parents.

Case definition

Upper respiratory tract infection (URTI) was diagnosed in the presence of characteristic signs or symptoms of rhinitis, pharyngitis, conjunctivitis or acute otitis media. Children with further signs or symptoms of pneumonia, bronchitis or bronchiolitis were diagnosed to have lower respiratory tract infection (LRTI).

Laboratory investigations

NP specimens were placed into transport medium (0.9% sterile sodium-chloride solution), sent to the laboratory immediately and stored at 4°C until PCR was performed (within 48 h). Nucleic acid for RT-PCR was extracted from a 140-μl nasopharyngeal sample with a commercial kit (QIAamp Viral RNA mini Kit, QIAGEN AG, Basel, Switzerland) according to the manufacturer's instructions.

RT-PCR for hMPV was performed by use of a one-tube reaction (Titan One Tube RT-PCR System, Roche Diagnostics GmbH Mannheim, Germany) according to the manufacturer's instructions. Briefly, a primer pair comprising hMPV L-L Hex labeled (5′ CAT GCC CAC TAT AAA AGG TCA G 3′) and hMPV L-R1 (5′ CAC CCC AGT CTT TCT TGA AA 3′), amplifying a fragment of 170 bp in the polymerase gene as described by van den Hoogen et al. [37], was used. In addition, a second primer pair hMPV N-L1 (5′ GCA TGC TAT ATT AAA AGA GTC TCA 3′) and hMPV N-R FAM labeled (5′ ATC TCA GCA GCA TAT TTG TA 3′) was used for amplification of a highly conserved fragment of 157 bp in the nucleoprotein gene. These primers were slightly modified based on those

previously described by Maertzdorf at al. [24]. PCR products then were subjected to capillary electrophoresis on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Amplified fragments were identified according to fluorescent color (corresponding to the labeled primers) and fragment size within the electropherogram.

As a positive control, RNA of hMPV (subtype A2, according to strain Canada 97/83) was used in every run of PCR (kindly provided by Prof. Dr. D. Neumann-Haefelin, Department of Medical Microbiology and Hygiene, University of Freiburg, Germany). The hMPV was considered present if either hMPV N gene or hMPV L gene or both could be detected.

The two hMPV RT-PCRs were integrated in the existing in-house RT-multiplex PCR as follows: primers for the hMPV N gene were combined with those for RSV, influenza viruses A and B (block 1), and primers for HMPV L gene were combined with those for adenoviruses and parainfluenza type 1 and type 3 viruses (block 2) [6, 13, 20, 26, 38]. RNA of RNA viruses was transcribed to cDNA before PCR was performed. PCR techniques were not changed over the course of the study period.

When specifically ordered by the physician, a separate PCR for the detection of *Mycoplasma pneumoniae* was also performed as described previously by Ieven et al. [17].

Statistical methods

Data were entered into a database, and statistical analyses were performed using SPSS (version 13.0, SPSS, Inc., Chicago, IL). Clinical characteristics and laboratory variables were compared by Student's t-test, Mann-Whitney U test, Fisher's exact test, chi squared test or odds ratio analysis as appropriate. A two-sided P value of <0.05 was considered significant.

Results

Epidemiological characteristics

During the two winter seasons from 11 October 2004 to 28 February 2006, a total of 2,582 NP specimens were received by our laboratory for PCR tests to detect respiratory pathogens. Based on the physicians' orders for the detection of viruses from multiplex-PCR blocks 1 and 2, 1,500 (58%) of 2,582 specimens were tested for hMPV: 1,499 (58%) for the hMPV N gene and 1,181 (46%) for the hMPV L gene (1,180 for N gene *and* L gene, 319 for N gene only and 1 for L gene only). Infection with hMPV was detected in 75 (5%) of 1,500 children, where 74 (4.9%) of 1,499 tested specimens were positive for hMPV N gene and 29 (2.5%) of 1,181 for hMPV L gene (p<0.01). Thus, PCR
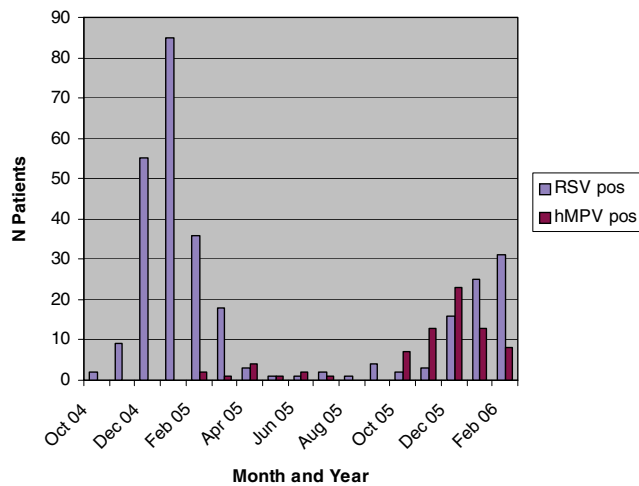
Fig. 1 Numbers and seasonal distribution of RSV and hMPV detected in nasopharyngeal aspirates from patients with respiratory tract infections

for L gene only contributed a single case that would not have been identified by use of N gene PCR primers.

Informed consent and clinical data could be obtained from the parents of 74 (99%) of 75 children infected with hMPV. Whereas only 3 of 691 (0.4%) NP specimens tested for hMPV in the first winter season of this study (October 2004 to March 2005) were positive, the proportion increased to 3.2% between April and September 2005 (8

of 248 NP specimens positive), and further increased during the following winter season to 11% (64 hMPV positives among 560 NP specimens). The increase in the proportion of hMPV started in October 2006, reached its peak in December and gradually declined over the next 2 months (Fig. 1).

RSV was detected in 294 (19.5%) of 1,505 specimens during the same study period with a higher prevalence during the winter season 2004/2005 compared to winter season 2005/2006 (Fig. 1). Overall, hMPV and RSV were detected in 14% and 54%, respectively, of all 520 samples positive for ≥1 respiratory virus by PCR during the study period (Fig. 2).

Five (7%) of the 75 hMPV-infected patients were documented to be coinfected with other respiratory pathogens: one (1%) with RSV and four (11%) with *M. pneumoniae* (of 36 NP specimens tested for both *M. pneumoniae* and hMPV).

Clinical characteristics

Patients with hMPV infections were significantly older (mean age 32 months, median 17, interquartile range: 7–44) than RSV-positive patients (mean age 16 months, median 9, interquartile range: 3–17; p=0.001). Specifically, 29 (39%) of 75 hMPV infections occurred in infants compared with 183 (62%) of 294 RSV infections (p<0.001).
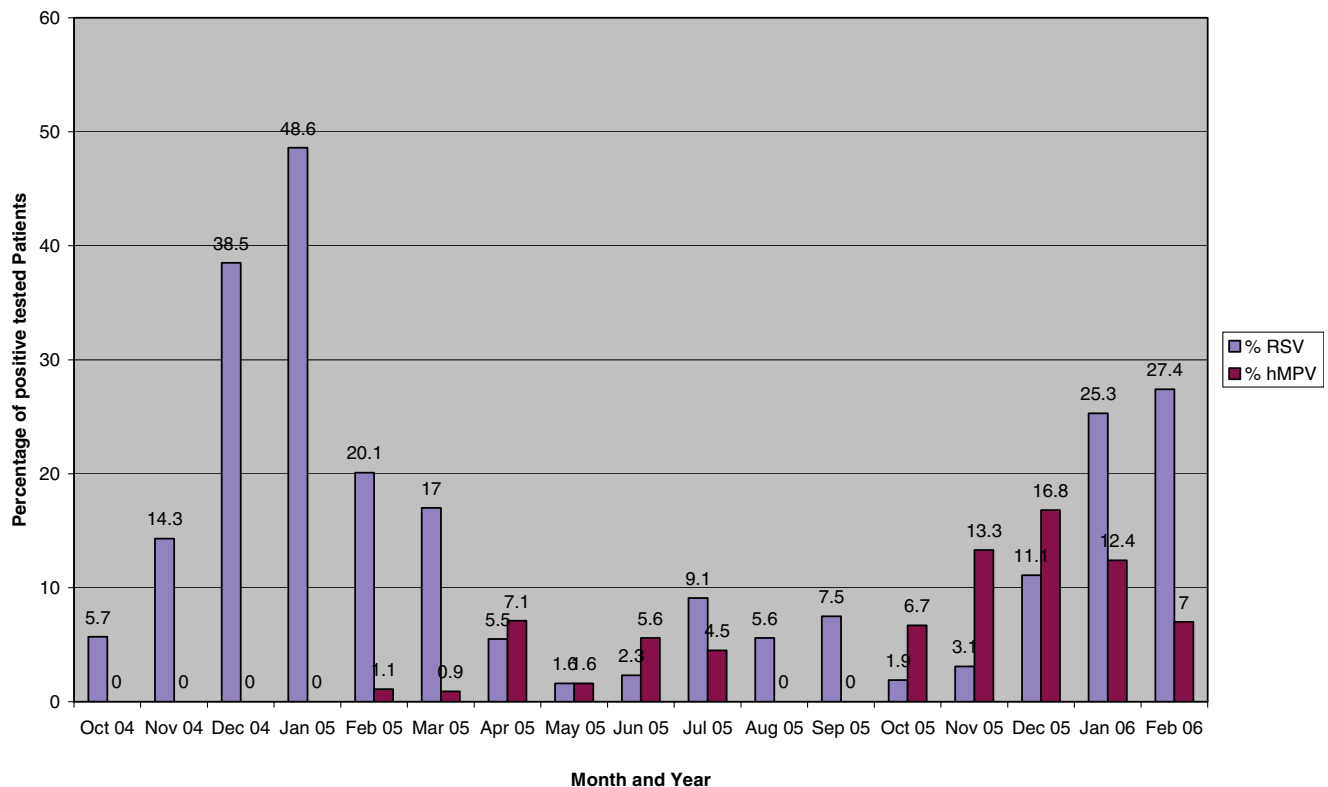

Fig. 2 Percentage of nasopharyngeal aspirates from patients with respiratory tract infections that tested positive for RSV and hMPV by study month

Male predominance was more pronounced among children with hMPV infections compared to those with RSV infections (63% versus 55%), but this difference was not significant.

Of 75 hMPV-positive children, 34 (45%) were hospitalized (RSV: 158 of 294; 54%; p=0.19), and 6 (18%) of them required intensive-care treatment because of imminent respiratory failure (RSV: 13 of 158; 8%; p=0.095). The remaining 41 patients were treated as outpatients. Of the six patients requiring intensive care, two had co-infections of hMPV and a further respiratory pathogen: one 17.8-year-old girl with trisomy 21, congenital heart disease and infection-associated hepatitis and myocarditis was co-infected with *M. pneumoniae*; she needed mechanical ventilation for 8 days and supportive oxygen therapy for 24 consecutive days. Furthermore, a 1-month-old boy was co-infected with RSV and also recovered on intensive care treatment.

A summary of clinical characteristics of hMPV infections and a comparison between hospitalized children and outpatients are demonstrated in Table 1. Mean duration of hospitalization for community-acquired hMPV infections was 6.6±8.9 days (median 4, interquartile range 2–6). One patient acquired a nosocomial hMPV infection. He had a VACTERL association and developed respiratory symptoms on day 46 from which he recovered. Subsequently, however, he also acquired parainfluenza virus infection and finally died on day 106 due to his underlying condition. No other deaths in hMPV-positive patients occurred.

Dyspnea, need for oxygen supplementation and feeding difficulties or loss of appetite were the only characteristics that were more frequent among hospitalized children who also tended to be younger than outpatients. Most of the patients with hMPV infections had LRTI (n=57; 77%), and this was true for both in- and outpatients. Age distribution

Table 1 Comparison of clinical characteristics of hospitalized and outpatients with hMPV infections

Clinical characteristics	Total[a] n=75 (%)	Outpatients[a] n=41 (%)	Hospitalized patients; n=34 (%)	P value[b]	OR (95% CI)
Age in months (mean±SD)	31.7±38.9	35.6±38.7	27±39.3		Not applicable
Male gender	47/75 (63)	26/41 (63)	21/34 (62)		1.07 (0.42–2.75)
Prematurity	8/61[c] (13)	4/27[c] (15)	4/34 (12)		0.77 (0.17–3.40)
Body temperature 37.1–38.4°C	17/74 (23)	9/40 (23)	8/34 (24)		Not applicable
≥38.5–38.9°C	14/74 (19)	12/40 (30)	2/34 (6)		Not applicable
≥39°C	43/74 (58)	19/40 (48)	24/34 (71)		Not applicable
Cough	73/74 (99)	39/40 (98)	34/34 (100)		Not applicable
Dyspnea	34/74 (46)	11/40 (28)	23/34 (68)	0.001	5.51 (2.03–14.97)
Wheezing	34/74 (46)	15/40 (36)	23/34 (68)		2.11 (0.83–5.36)
URTI	65/74 (88)	33/40 (83)	32/34 (94)		3.39 (0.66–17.58)
- Conjunctivitis	7/74 (9)	2/40 (5)	5/34 (15)		3.28 (0.59–18.10)
- Rhinitis	58/74 (78)	29/40 (73)	29/34 (85)		2.20 (0.68–7.13)
- Pharyngitis	34/74 (46)	16/40 (40)	18/34 (53)		1.69 (0.67–4.25)
- AOM	10/74 (14)	7/40 (18)	3/34 (9)		0.46 (0.10–1.92)
LRTI[d]	57/74 (77)	30/40 (75)	27/34 (79)		1.29 (0.43–3.85)
- Pneumonia	25/74 (34)	10/40 (25)	15/34 (44)		2.37 (0.88–6.34)
- Bronchitis	34/74 (46)	21/40 (51)	13/34 (38)		0.56 (0.22–1.42)
- Bronchiolitis	13/74 (18)	5/40 (13)	8/34 (24)		2.15 (0.63–7.35)
- Croup	5/74 (8)	2/40 (5)	3/34 (9)		1.84 (0.29–11.71)
Exanthema	8/74 (11)	3/40 (8)	5/34 (15)		2.13 (0.47–9.64)
Feeding difficulties/loss of appetite	26/74 (35)	9/40 (23)	17/34 (50)	0.014	3.44 (1.26–9.38)
Vomiting	25/74 (34)	13/40 (33)	12/34 (35)		1.13 (0.43–2.97)
Concomitant diarrhea	19/74 (26)	14/40 (35)	5/34 (15)	0.046	0.32 (0.10–1.01)
Treatment					
- Antibiotics	38/74 (51)	19/40 (48)	19/34 (56)		1.40 (0.56–3.51)
- Oral steroids	9/74 (12)	1/40 (3)	8/34 (24)	0.017	12.0 (1.42–101.7)
- Inhalative therapy	27/74 (36)	7/40 (18)	20/34 (59)	0.001	6.73 (2.32–19.5)
- Bronchodilatator	20/74 (27)	5/40 (13)	15/34 (44)	0.003	Not applicable
- Bronchodilatator/steroids	7/74 (9)	2/40 (5)	5/34 (15)	0.003	Not applicable
- Oxygen therapy	13/74 (18)	0/40 (0)	13/34 (38)	<0.0001	Not applicable

[a] Data on most characteristics are missing for one outpatient due to lack of parental consent
[b] If <0.05
[c] Data on 14 patients missing due to lack of documentation (n=13) or lack of parental consent (n=1)
[d] More than one diagnosis of LRTI was made in some patients

was similar between patients with LRTI and those with URTI only (data not shown). Interestingly, though, children with pneumonia were significantly older than those with other manifestations of respiratory tract disease (mean 46.9± 47.2 months and 24.5±32.2 months, respectively; p=0.04).

Three children-17, 19 and 36 months old-presented with a febrile convulsion, and one further patient experienced a relapse of nephrotic syndrom while suffering from hMPV infection. Twenty-two (30%) of 74 hMPV infected children had various forms of underlying chronic disease and 15 (68%) of them were hospitalized compared with 19 (37%) of 52 primary healthy children (p=0.03).

Discussion

Human metapneumovirus, a member of the *Paramyxoviridiae* family, is a single-stranded negative-sense RNA virus that was first described by Dutch investigators, who isolated the virus from young children with RTI [36]. Here we report the first description of a large number of hMPV infections in children in Switzerland and provide a detailed description of epidemiological and clinical characteristics.

During the 2-year study period the detection rate steadily increased from 0.4% (months 1–6) to 3.2% (months 7–12) and finally reached a proportion as high as 11% of tested NP samples being hMPV positive (months 13–17). Whether this represents a pattern of biannual major epidemics similar to those observed with RSV remains to be seen [2, 10, 39]. Previous reports of hMPV infections from various countries have described a characteristic seasonality with peaks during the cold seasons [9, 15, 18, 34, 42], but none of these studies was performed over a prolonged period of time. Similarly, we observed an impressive peak during winter 2005/2006, and we will continue our observations in order to better define the seasonality of hMPV infections in the future.

The overall detection rate for hMPV in this study was 5%, which is in the lower range of rates that have been detected among children with acute RTIs in previous investigations from Europe, North and South America, South Africa, Australia and Asia, most of them restricted to hospitalized patients, i.e., 5 to 16% [8, 12, 19, 21, 27, 30, 31, 42]. Studies with detection rates in the upper range usually were restricted to winter seasons when peaks of infections occur. In contrast, those with rates in the lower range frequently also covered summer months (which necessarily leads to a lower percentage of detection), like our study, which was uninterrupted for 17 consecutive months.

Although no viral cultures were performed to assess the overall sensitivity of PCR tests in this study, PCR based on N gene primers was more sensitive than PCR based on L gene primers. In a comparative evaluation of real-time PCR assays for the detection of hMPV, N and L gene primers were shown to be most suitable, and sensitivity of N gene primers was superior to that of L gene primers, which is in accordance with our findings [7]. Given the fact that the additional use of L gene primers contributed only minimally to the overall sensitivity of PCR for hMPV in our study, it appears that the use of N gene primers alone is sufficient.

The presenting signs and symptoms of hMPV infection are reminiscent of those associated with RSV [25]. However, hMPV-infected patients were significantly older than RSV-infected patients in this study, which is consistent with findings in the literature [30, 37, 42, 43]. Moreover, despite the fact that both viruses mainly circulate during the winter season, we identified only a single patient with dual hMPV and RSV infection. This underlines the notion that both viruses independently cause similar infections [37].

When comparing the clinical presentation of hospitalized hMPV-infected patients with those of outpatients, hospitalized patients tended to be younger and more frequently suffered from dyspnea and feeding difficulties or loss of appetite. Interestingly, 77% of hMPV-infected patients suffered from LRTI. This was not different between hospitalized and outpatients, which may be explained by the retrospective design of our study where outpatients with more serious disease probably had been selected for diagnostic testing. However, wheezing tended to be more frequent among hospitalized children (68%). Consequently, 38% of them needed oxygen supplementation (compared to none of the outpatients), and steroids and bronchodilators were also used more frequently in hospitalized children than in outpatients. Although this was a retrospective analysis open to the introduction of bias, these differences are quite plausible.

Approximately 50% of patients received antibiotics during some stage of their illness, and this rate appears to be high. However, in most instances antibiotic treatment was prompted by concomitant pneumonia or acute otitis media where secondary bacterial infections apparently were suspected and/or the presence of hMPV infection was not yet known. Similarly, 60% of hospitalized children with hMPV infection were treated with antibiotics in the Netherlands, a country with a restrictive policy for the use of antibiotics like in Switzerland [37].

Thirty percent of hMPV-infected patients had chronic underlying diseases, and 11% had a history of prematurity, similar to previous observations [4, 12, 37, 40]. This may be an indication for increased susceptibility in these hosts and/ or an artifact explained by the fact that physicians are more likely to order diagnostic tests in these high-risk patients.

There are conflicting results concerning whether dual infections by hMPV and other respiratory tract viruses, mainly RSV, are associated with a more severe disease than that observed with hMPV as the single etiological cause of disease. Semple et al. reported a tenfold increase in the

relative risk of admission to a pediatric intensive-care unit for mechanical ventilation due to severe bronchiolitis in patients with dual infection with hMPV and RSV [35]. Similarly, dual infections with hMPV and RSV were found in 70% of infants with severe bronchiolitis admitted to a pediatric intensive-care unit for mechanical ventilation [16]. This is further supported by other recent data mainly in children less than 3 years of age [14, 22]. In contrast, other studies have failed to demonstrate more serious disease with dual infections [5, 15, 23, 40, 41, 43]. Although we only found a single patient with hMPV and RSV co-infection, this infant required intensive care treatment. Larger studies are needed to elucidate whether-and if so by which mechanisms-hMPV and RSV co-infection leads to a more serious course of respiratory disease or not.

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