Eur J Med Res (2006) 11: 329-335

Excretion Patterns of Human Metapneumovirus and Respiratory Syncytial Virus among Young Children

M.-L. von Linstow[1], J. Eugen-Olsen[2], A. Koch[3], T. N. Winther[1], H. Westh[4], B. Høgh[1]

[1]Department of Paediatrics, [2]Clinical Research Unit, [4]Department of Microbiology, Hvidovre Hospital, University of Copenhagen, Denmark
[3]Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark

Abstract
Background: As respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) cause serious respiratory tract infections, the routes of transmission of these viruses are important to elucidate. We examined the modes of virus shedding and shedding duration of RSV and hMPV in young children.
Methods: From each child in a group of 44 children (37 RSV-positive, 6 hMPV-positive, and 1 co-infected child), aged between 0.5-38 months, hospitalised at Hvidovre Hospital, Copenhagen, Denmark, one nasopharyngeal aspirate (NPA), saliva, urine, and faeces sample were collected at inclusion and weekly in a three-week period. Sweat and blood samples were obtained at inclusion. The presence of RSV and hMPV RNA was detected using real-time RT-PCR.
Results: We detected RSV RNA in 28 saliva specimens, 5 stool samples, and 3 sweat samples. hMPV RNA was detected in one saliva specimen and two sweat samples. Four of the five children shedding RNA in faeces had diarrhoea and children shedding RNA in sweat were either less than five weeks of age or had a chronic lung disease. RSV and hMPV RNA was shed in nasal secretions for a median of 11.5 and 5.0 days respectively (p=0.001). More than 75% of the family members of the infected children showed to have an upper respiratory tract infection when following up.
Conclusion: Viral RNA was present in nasal secretions, saliva, sweat, and faeces, but whether or not the virions were infectious and constitute a potential mode of transmission remains to be shown in future studies.

Key words: Human metapneumovirus, respiratory syncytial virus, shedding, excretion, children

Abbreviations: RSV: respiratory syncytial virus, hMPV: human metapneumovirus, NPA: nasopharyngeal aspirate, RT-PCR: reverse transcription-polymerase chain reaction, IQR: inter-quartile range, PBMC: peripheral blood mononuclear cells

Introduction

Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infection among young children. Human metapneumovirus (hMPV) is a recently discovered respiratory virus belonging to the Paramyxoviridae family [1]. hMPV causes a respiratory illness with clinical symptoms that resembles those seen in connection with RSV infection. As both RSV and hMPV cause serious respiratory tract infection, the excretion patterns and routes of transmission of these viruses are important to elucidate.

RSV has tropism for epithelial cells and alveolar macrophages [2]. Little is known about the pathophysiology of hMPV infection, but hMPV appears to have tropism for the respiratory epithelium [3]. An animal study of hMPV infection in cynomolgus macaques showed that viral replication was restricted to the respiratory tract [4].

Until now it has been widely accepted that spread of RSV occurs through respiratory secretions only, either through large droplets of secretions or through direct contact with contaminated secretions [5]. Likewise, hMPV is only known to be shed in respiratory secretions. In contrast, other respiratory viruses such as the coronavirus causing severe acute respiratory syndrome have been detected in the intestinal tract and in sweat glands of the skin [6] and are excreted via faeces [7]. A study of the respiratory pathogen avian metapneumovirus in chickens showed live virus to be shed in faeces of non-vaccinated hens [8].

Spread of RSV is highly effective: exposure to an RSV-infected infant over two to four hours results in infection among 71% of exposed individuals, even though the infection is not airborne [5]. Similarly, hMPV also spreads effectively among exposed individuals [9].

The highly effective spread of RSV and hMPV and the findings of other respiratory viruses in various tissues and secretions let us to hypothesise that RSV and hMPV might be transmitted in secretions other than nasal secretions. The aim of this study was therefore to examine the modes of virus shedding and the shedding duration of RSV and hMPV in young children.

Materials and Methods

Study Population

The study population consisted of children who were admitted to the Department of Paediatrics, Hvidovre Hospital, Copenhagen, Denmark with an acute respiratory tract infection between 1 November 2003 and

30 April 2004, and who had either RSV or hMPV detected in a routine nasopharyngeal aspirate (NPA) by RT-PCR. Children whose parents did not speak or understand Danish were not included.

Methods

The parents of the children were contacted as soon as the result of the NPA test was available, either personally while the children were still hospitalised or by telephone if they had been discharged by the time of the test result. After the written informed consent of the parents, one NPA specimen, one saliva sample, one urine sample, and one stool sample was taken from each child at inclusion and again after 1, 2, and 3 weeks. In addition, one sweat sample was collected shortly after diagnosis. Samples were collected at the hospital and during weekly home visits by one of the authors (MLL) or a trained project-nurse. In regard to children who had blood samples taken due to their illness, extra blood samples were collected for the study.

Data Collection:

Parents were interviewed about their child's general health and the current illness during the home visits at week 1, 2, and 3. Questions included ethnicity, gestational age, household members, duration of breastfeeding, bedroom-sharing, day-care attendance, smoking in household, asthma or hay fever in the family, the child's previous diseases (pneumonia, asthmatic bronchitis, otitis media, atopic dermatitis, and chronic diseases), nature and duration of present symptoms (rhinitis, ear pain, ear discharge, cough, hoarseness, breathing difficulties, fast breathing, conjunctivitis, rash, anorexia, diarrhoea, vomiting, fatigue, and fever), medical treatment prior to hospitalisation, and related diseases in household members.

Medical records of all children were systematically reviewed and the following parameters were noted: duration of stay; clinical signs: tachypnoea, wheezing, chest indrawings, stridor, tachycardia, crepitation, rhonchi, and prolonged expiration; transcutaneous oxygen saturation; body temperature; blood gas values and infection parameters; chest x-ray; treatment; and diagnosis when discharged.

Specimen Collection:

NPA specimens were taken using a soft plastic-tube sucking secretions from the nasopharynx into a sterile 10 ml tube (Maersk Medical A/S). Afterwards, two ml of PBS buffer was suctioned through the plastic-tube and the secretions were collected in the sterile tube. At home visits, the same device connected to a foot pump (Ambu® Twin Pump, Ambu International A/S) was used.

Saliva was collected using the Oracol device (Malvern Medical Developments, Worchester, UK), which is a cylindrical polystyrene sponge attached to a plastic stick designed to be used as a toothbrush, that is rubbed against the gums until the sponge is wet. Two such devices were used for each saliva collection. The sponges were placed in the plastic tubes and centrifuged for 2 min. at 3600 rpm.

One to two ml blood was collected by a heal prick or from the cubital vein, centrifuged at 3000 rpm for 10 min., and divided into cells and plasma.

Sweat was collected by pilocarpine iontophoresis (Macroduct sweat collection system, Wescor Inc.). A disc of 0.5 % pilocarpine (Pilogel Disc, Wescor Inc.) was applied to the medial aspect of the forearm after washing thoroughly with sterile water. After stimulation with iontophoresis for five minutes the arm was again cleaned with sterile water and sweat was collected through a closed spiral microbore tube (Macroduct sweat collector, Wescor Inc.) which was placed on the stimulated area of the arm for 30 min. Sweat was transferred to a cryo tube and frozen immediately at -80 °C.

Urine was collected using a plastic urine collector bag attached to the outer genitalia and then transferred to a sterile 10 ml glass.

Faeces were collected from the nappy by a sterile spoon and placed into a stool transportation tube containing 3-4 ml of Stool Transportation And Recovery (S.T.A.R.) buffer (Roche Diagnostics, GmbH, Germany). The S.T.A.R buffer contains nucleases inactivators and permits storage and shipment of stool specimens for up to five days at 15-20 °C without compromising the stability of RNA. Prior to RNA extraction, the samples were prepared according to the manufacturer's instructions.

The parents were instructed in how to collect urine and stool samples from their child. The samples were collected on the same day or the day before our home visit and stored at 4 °C. If samples were not ready at our visits, they were sent by mail later the same day. As soon as the samples were received in the laboratory, they were frozen at -20 °C for later analyses.

LightCycler Reverse Transcription-polymerase Chain Reaction (RT-PCR) Assays for hMPV and RSV

RNA extraction: Nucleic acids were extracted from 100 μl of each NPA and saliva specimen and from 200 μl of each urine and faeces specimen using the MagNA Pure LC Total Nucleic Acid kit (Roche Diagnostics, GmbH, Germany) automated extraction system according to the manufacturer's instructions. Extracted nucleic acids (50 μl) were stored at -80 °C.

RNA from sweat and blood samples was extracted manually using the High Pure Viral RNA kit (Roche Diagnostics, GmbH, Germany). Before RNA extraction, sweat samples were diluted in a 50 μL PBS buffer.

hMPV RT-PCR: A single-tube real-time RT-PCR using the LightCycler instrument (Roche Diagnostics, GmbH) was performed using commercially synthesized (TIB MOLBIOL, Berlin, Germany) primers targeting the N gene and a fluorogenic endonuclease oligoprobe (TaqMan) for detection as previously described [10]. In addition, all samples were examined with another primer set targeting the N gene as published by Maertzdorf et al [11].

RSV RT-PCR: Similarly, the LightCycler was used for RSV RT-PCR using primers and fluorescence reso-

nance energy transfer (FRET) probes (TIB MOLBIOL, Berlin, Germany) for detection of RSV as described by Whiley et al [12].

In addition to the closed-tube design of these single-round real-time RT-PCR assays, standard precautions were taken throughout the PCR process to avoid possible cross-contamination and dUTP and UNGase were included in each PCR reaction to prevent carry-over contamination. Furthermore, positive and negative LC-RT-PCR controls were included in each run. In addition to melting curve analysis, the PCR products of the RSV- and hMPV-positive samples were analysed by electrophoresis in a 3% agarose gel and stained with ethidium bromide. The PCR products were of the expected size, 170 bp and 213 bp respectively. As a further control of the specificity of the RT-PCR reactions, PCR products were sequenced in both directions using the BigDye terminator cycle sequencing Kit version 3.1 (Applied Biosystems) and the same primers as applied in the detection PCR. The sequencing reactions were run on an ABIprism 3100 Genetic Analyser (Applied Biosystems). The obtained sequences were analysed using the program Blast (http://www.ncbi.nlm.nih.gov/BLAST/).

RT-PCR routine diagnostics for RSV and hMPV were performed daily except on weekends and public holidays. On weekends and public holidays, Directigen™ RSV (Becton Dickinson, USA) was used while hMPV samples were analysed on the next weekday. All follow-up samples were analysed by RT-PCR.

Statistical Methods

Differences in proportions between RSV and hMPV infected children were tested using the chi-square test or, when appropriate, Fisher's exact test. Differences in median values between the groups were tested using the non-parametric Mann-Whitney test. Shedding duration for RSV and hMPV was estimated by Kaplan-Meyer curves and differences were evaluated by a log rank test. Time of shedding was approximated as the midpoint between the time of the last positive test and the time of the first negative test afterwards. Children whose last NPA sample was positive were censored at that time. Data were analysed using the SPSS software version 12.0 for Windows. A p-value <0.05 was considered significant.

The Ethics Committee of Frederiksberg, Copenhagen, Denmark, approved the study.

Results

Study Population

During the winter of 2003/2004, 292 children admitted to hospital had a routine NPA taken on suspicion of RSV infection. Thirteen (4.5%) of these children were hMPV-positive by RT-PCR. Of the 292 NPAs, 231 were analysed for RSV by RT-PCR and 113 (49%) of these were RSV-positive. Due to working procedures during weekends, 61 samples were analysed for RSV by ELISA only. We were able to contact 90 parents of the 126 children with a positive test result. Twelve children were excluded as their parents did not speak sufficient Danish, and the parents of 24 of the children did not want to participate. Five children lived outside the admission area of the hospital.

Thus, 49 children were included in this study (39 RSV-positive children, 9 hMPV-positive children and 1 RSV/hMPV co-infected child). One hMPV-positive child was excluded as duplicate testing with RT-PCR showed a false positive result. Two RSV and 2 hMPV-positive children dropped out within the first week, leaving 37 RSV-positive children, 6 hMPV-positive children, and the co-infected child for analyses. One of the children was included twice, first with an RSV infection and three weeks later with an hMPV infection. Characteristics of the children are given in Table 1. Participants and non-participants among the 126 children with a positive test result did not differ significantly according to median age and gender (4.2 months vs. 4.3 months and 67% boys vs. 61% boys respectively).

Table 1. Characteristics of 38 RSV-positive children and 7 hMPV-positive children, including one RSV/hMPV co-infected child*. Differences between the two groups are all non-significant.

	RSV+ (N=38)	**hMPV+ (N=7)**
Non-Scandinavian (%)	7 (18)	2 (29)
Male (%)	27 (71)	4 (57)
Age** (mo)	3.7 (0.5-32.9)	7.8 (1.4-38.1)
Duration of symptoms prior to hospitalisation** (days)	4 (0-17)	4 (4-7)
Hospitalisation time** (days)	5 (1-14)	2.5 (1-7)
Gestational age** (wk)	40 (29-42)	39 (28-42)
Chronic disease*** (%)	4 (11)	2 (29)
Household size**	4 (2-6)	4 (3-4)
No. of siblings**	1 (0-3)	1 (0-1)
Smoking in household (%)	8 (21)	1 (14)

* The RSV/hMPV co-infected child was a non-Scandinavian boy aged 10 months with asthma. Gestational age: 39 weeks. He had had symptoms for four days when hospitalised and stayed for three days. The non-smoking home consisted of four persons including one sibling.
**Median values (range)
*** Three RSV+ children had asthma and one had cerebral palsy. One hMPV+ child had asthma and one had bronchopulmonal dysplasia and physical retardation.

Excretion of Viral RNA

Home visits and specimen collection were performed at a mean of 8.3 days (range 4-13 days), 15.4 days (range 12-20 days), and 21.9 days (range 18-27 days) after admission to hospital.

A total of 723 specimens were collected during the study period. This included 175 NPAs, 166 saliva spec-

Table 2. Excretion of RSV and hMPV RNA in different sample material detected by RT-PCR. Days are reported as the time from the diagnostic NPA at day 0.

Patient No. RSV pos.	NPA day 0	NPA day 5-13	NPA day 12-20	NPA day 18-27	Saliva** day 0-6	Faeces day 0-6	Sweat day 0-5	Urine day 0-6	Blood* day 0-7
4	+	-	+	-	+	+	nd	-	-
5	+	+	-	+	+	-	-	-	-
6	+	+	-	-	+	+	-	-	nd
7	+	-	+	-	-	-	-	-	-
8	+	+	-	-	+	-	nd	nd	nd
9	+	-	-	nd	+	-	-	-	nd
10	+	+	-	-	+	-	-	-	-
11	+	-	+	-	+	-	-	-	-
12	+	+	+	+	-	-	-	-	-
13	+	-	-	-	+	-	-	-	-
14	+	-	nd	nd	+	+	+	-	-
15	+	+	+	+	-	-	-	-	-
16	+	-	-	-	+	-	-	-	-
17	+	+	-	-	+	-	-	-	-
18	+	+	-	-	-	-	-	-	nd
19	+	+	-	-	-	-	-	-	nd
20	+	-	nd	nd	-	-	-	nd	nd
22	+	-	-	-	+	+	-	-	nd
23	+	+	+	-	-	-	-	-	nd
25	+	+	-	-	+	-	-	-	nd
26	+	-	-	-	-	-	-	-	nd
27	+	+	-	+	-	-	-	-	nd
28	+	-	-	+	+	-	+	-	nd
29	+	-	-	-	+	-	-	-	-
30	+	+	-	nd	-	-	-	-	-
31	+	+	-	-	-	-	-	-	nd
32	+	-	-	-	-	-	-	-	-
34	+	-	+	+	+	-	-	-	-
35	+	-	-	-	+	-	nd	-	-
36	+	+	-	-	+	-	-	nd	nd
39	+	-	+	-	-	-	-	-	nd
41	+	-	+	+	+	+	-	-	nd
42	+	+	-	-	+	-	+	nd	-
43	+	+	-	-	+	-	-	-	nd
46	+	+	-	-	-	-	-	-	-
47	+	-	nd	-	+	-	-	-	nd
48	+	+	nd	-	nd	nd	-	nd	nd
49	+	+	-	-	+	-	-	-	nd

Patient No. hMPV pos.	NPA day 0	NPA day 4-10	NPA day 12-17	NPA day 20-24	Saliva*** day 1-4	Faeces day 0-4	Sweat day 1-4	Urine day 1-4	Blood* day 0
21	+	-	-	-	-	-	+	-	nd
24	+	+	-	-	-	-	-	-	-
28	+	-	-	-	-	-	+	-	nd
38	+	+	-	-	-	-	-	-	nd
40	+	-	-	-	-	-	-	-	nd
44	+	+	-	-	+	nd	-	nd	-
45	+	-	-	nd	-	-	-	-	-

+ positive test result, - negative test result, nd: not done
* Two additional blood samples from two hMPV-positive infants, who dropped out of the study, were negative.
** Three patients shed RSV in saliva after 7and 8 days, one after 14 days and one after 19 days.
*** One additional saliva sample from an hMPV-positive child who dropped out (patient no. 2) was hMPV positive at day 0.

imens, 41 sweat samples, 155 urine samples, 165 faeces specimens, and 21 blood samples.

In saliva samples we detected RSV and hMPV RNA from 26/38 (68%) and 1/7 (14%) of the children respectively (Table 2). 24 (89%) of these samples were taken within the first six days after diagnosis.

RSV RNA was found in five stool samples from five different children. All positive samples were taken

within two days of the diagnostic NPA and four of the children had diarrhoea. hMPV RNA was not detected in stools.

Three RSV-positive children (8%) and two hMPV-positive children (29%) shed viral RNA in sweat including the child with co-infection, who shed both RSV and hMPV in sweat. Positive samples were taken within three days of the first positive NPA. The amount of sweat collected from the children varied from <5 μL to more than 100 μL. Due to the small amount of sweat, successful sequencing was obtained for one of the RSV positive samples only (RSV from the hMPV co-infected child). One of the children shedding RSV in sweat also shed RSV in faeces (a 14-day-old girl). The other children shedding RSV RNA in sweat were an otherwise healthy 33-day-old girl and a boy of 10 months who had experienced frequent episodes of severe wheezing since he was one month old. He was co-infected with hMPV and shed hMPV in sweat too. The other child shedding hMPV in sweat was a 3-year-old girl, prematurely born (29th wk), who had bronchopulmonal dysplasia, asthma, and frequent episodes of lower respiratory tract infections.

We did not detect viral RNA in urine or blood samples.

Shedding Duration

Duration of viral RNA shedding in nasal secretions varied from less than one week to more than three weeks after admission to hospital. As illustrated in Table 2, nine of the children shedding RSV RNA after two or three weeks had a negative RSV PCR test result in between. Six of these children had respiratory symptoms by the time of the negative sample collection. Further melting point temperature analyses of NPAs from six of these children showed that they were infected with the same subtype of RSV (A or B) in all their positive samples. Assuming that these nine children shed RSV until the last positive sample, the median duration of RSV shedding was 11.5 days (Inter-quartile range (IQR) 6.5-18.5) while the median duration of hMPV shedding was 5.0 days (IQR 3.5-10.0) (p = 0.001, Fig. 1).

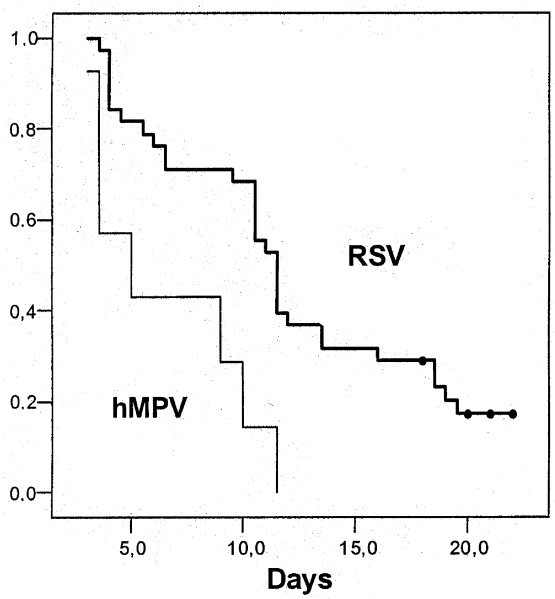

Fig. 1. Kaplan Meyer analysis of shedding duration of RSV and hMPV RNA in nasopharyngeal aspirate specimens. RSV: median 11.5 days (IQR 6.5-18.5), hMPV: median 5.0 days (IQR 3.5-10.0), p = 0.001
•:RSV-censored samples

Symptoms and Clinical Signs

The children showed a broad spectrum of symptoms and clinical signs, which did not differ significantly between the RSV and hMPV positive groups (Table 3).

Seven days after hospitalisation, the parents reported that their children had suffered from 5 to 13 (median 9) different symptoms. Three weeks after hospitalisation, 54% of the RSV-positive children and 33% of the hMPV-positive children (p = 0.3) showed respiratory symptoms.

Children shedding RSV in stools had significantly more diarrhoea (4/5 vs. 8/33, p<0.05) and less rhonchi (0/5 vs. 20/30, p<0.01) and prolonged expiration (0/3 vs. 20/26, p<0.05) than the remaining RSV-positive children, while there was no difference with respect to other symptoms (data not shown). The duration and severity of symptoms, length of hospital stay, and viral shedding in the NPAs did not differ between the children shedding RNA in faeces and sweat and the whole study group.

Table 3. Selected symptoms from within the first week of illness and clinical signs during hospitalisation among 38 RSV-positive and 7 hMPV-positive children, including one co-infected child. Ear pain, ear discharge, hoarseness, and fatigue are not included in the table as very few of the children showed such symptoms. Stridor and tachycardia are not included in the table due to infrequent registration. Differences between the two groups are all non-significant.

	No./N (%) RSV+	hMPV+
Respiratory tract		
Rhinitis	38/38 (100)	6/6 (100)
Cough	38/38 (100)	6/6 (100)
Tachypnoea	30/35 (86)	5/6 (83)
Wheezing	16/27 (59)	2/2 (100)
Chest indrawings	28/36 (78)	5/5 (100)
Crepitation	20/34 (59)	2/5 (40)
Rhonchi	20/35 (57)	4/5 (80)
Prolonged expiration	20/29 (69)	3/3 (100)
Gastrointestinal tract		
Anorexia	34/38 (89)	6/6 (100)
Vomiting	14/38 (37)	1/6 (17)
Diarrhoea	12/38 (32)	2/6 (33)
Other symptoms and signs		
Conjunctivitis	13/38 (34)	4/6 (67)
Rash	4/38 (11)	0/6 (0)
Fever	33/38 (87)	6/6 (100)

Spread in Families

More than three quarters of the family members of the infected children reported symptoms of upper respiratory tract infection in a four-week period from one week prior to the child's illness through the three weeks of follow-up (78% vs. 82% in RSV and hMPV-positive families respectively). In 37% of the RSV and 33% of the hMPV-infected families, all family members developed respiratory symptoms during the follow-up period, and in only two families (both with a RSV-positive child), no other family members developed symptoms. Families of children shedding viral RNA in sweat or faeces experienced the same amount of illness as the remaining families. In families with more than one child, an older sibling was the first person to show symptoms in more than two thirds of the cases (75% for RSV and 60% for hMPV). In 21% of the cases, the patient was the first to show symptoms, and in 25% of the cases, a parent apparently introduced the virus to the family.

Discussion

This study describes the excretion of RSV and hMPV RNA in different secretions, which have not previously been reported as possible modes of transmission. We detected RSV RNA in nasal secretions, saliva, sweat, and faeces, and hMPV RNA in nasal secretions, saliva, and sweat. The finding of viral RNA in the saliva is not surprising due to the close contact with the nasopharynx. RSV RNA was found in faeces specimens from five children of which four had diarrhoea. An explanation for the finding of RSV RNA in stools might be that the children are likely to have swallowed respiratory secretions containing RSV. We did not perform viral culture analyses of the stools, but the isolation of viral RNA after transportation through the entire gastrointestinal tract without being degraded indicates that this viral RNA was encapsidated into viral particles and may therefore be infectious.

We detected RSV and hMPV RNA in sweat from four children of which one was co-infected and shed both RSV and hMPV RNA. A short viraemic phase would allow the virus to spread to the sweat glands to be excreted or to replicate. Other groups have detected RSV and hMPV RNA in blood [13, 14]. Some have been able to detect RSV RNA only in peripheral blood mononuclear cells (PBMC) and not in serum [15, 16]. Recently, rhinovirus viraemia has been described to occur during the first 24 hours from symptom initiation in normal children with a respiratory infection [17]. Excretion of viral RNA in sweat was limited to children of less than five weeks or children with a chronic lung disease, indicating that an immature or defective immune response makes it easier for virus to spread from the upper respiratory tract.

It is only possible to collect a relatively small amount of sweat from young children, and freezing and thawing undiluted samples makes the RNA very sensitive to degradation. This made it difficult to reproduce our findings by RT-PCR and due to lack of material we were not able to perform cell culture analyses and only succeeded in sequencing one sample. However, this one sample proved to be RSV, supporting the positive findings in the four other specimens.

We found the median shedding time of RSV to be 11.5 days. The duration of hMPV shedding was shorter with a median of five days. Shedding in stools only occurred within the first week of illness. In the 1970s, Hall and colleagues carried out several studies of RSV transmission focusing on RSV shedding in nasal secretions [5, 18-20]. The shedding duration of RSV in children with a lower respiratory tract infection was found to be a mean of 8.4 days measured by cell culture [18]. No similar studies have been published for hMPV. Our results are based on findings by RT-PCR, which may be more sensitive than cell culture explaining the longer shedding period in our study. However, our Kaplan-Meyer estimate of shedding time may be somewhat rough as we used the midpoint between the time of the last positive test and the time of the first following negative test as the estimated time for cessation of shedding for each child. There were nine children who presented a negative sample in between two positive ones. While new infection cannot be entirely ruled out, we believe the most likely explanation is collection of insufficient sample material, as in some cases, in particular at home visits, we could only obtain a small amount of secretion from the children's nasopharynx.

Hall found that RSV infected 44% of the families and 22% of the family members of RSV-infected children verified by nose and throat specimens [18] and Bosis et al found that 4.7% of households with RSV-positive children reported a disease similar to that of the infected child. The similar number for hMPV-positive families was 12.5% [9].

In our study, more than 75% of the members of the infected children's families had an upper respiratory tract infection in the period ranging from one week prior to the child's illness through the three weeks of follow-up, most of them reporting symptoms before the child. As we did not obtain specimens from family members, this number might be an overestimate of the true infection rate of RSV and hMPV in family members. However, the findings indicate that, in most cases, a family member may have infected the child.

In conclusion, viral RNA was present in nasal secretions, saliva, sweat, and faeces. The shedding period in respiratory secretions was longer for RSV (11.5 days) than for hMPV (5.0 days). Whether the virions isolated from sweat and faeces were infectious and represent a mode of transmission remains to be shown in future studies.

Acknowledgements: We are grateful for the support from the parents of the children. We thank Karina Larsen who assisted in collecting the samples and Anja Stausgaard for her skilful technical assistance. We thank Klaus Larsen and Jan Wohlfahrt for useful discussions on the statistical analyses.

This study was supported by grants from the Pharmacists' Foundation, the Augustinus Foundation, H:S Central Research Fund, and the Research Fund of Queen Louise's Children's Hospital.

REFERENCES:

1. Van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA, Osterhaus AD. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med. 2001 Jun;7(6):719-24.
2. Easton AJ, Domachowske JB, Rosenberg HF. Animal pneumoviruses: molecular genetics and pathogenesis. Clin Microbiol Rev. 2004 Apr;17(2):390-412.
3. Vargas SO, Kozakewich HP, Perez-Atayde AR, McAdam AJ. Pathology of human metapneumovirus: Insights into the pathogenesis of a new identified respiratory virus. Pediatr Dev Pathol. 2004 Sep-Oct;7(5):478-86.
4. Kuiken T, van den Hoogen BG, van Riel DA, Laman JD, van Amerongen G, Sprong L, Fouchier RA, Osterhaus AD. Experimental human metapneumovirus infection of cynomolgus macaques (Macaca fascicularis) results in virus replication in ciliated epithelial cells and pneumocytes with associated lesions throughout the respiratory tract. Am J Pathol. 2004 Jun;164(6):1893-900.
5. Hall CB, Douglas RG. Modes of transmission of respiratory syncytial virus. J Pediatr. 1981 Jul;99(1):100-3.
6. Ding Y, He L, Zhang Q, Huang Z, Che X, Hou J, Wang H, Shen H, Qiu L, Li Z, Geng J, Cai J, Han H, Li X, Kang W, Weng D, Liang P, Jiang S. Organ distribution of severe respiratory syndrome (SARS) associated Coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. J Pathol. 2004 Jun;203(2):622-30.
7. Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, Rabena H, Panning M, Kolesnikova L, Fouchier RA, Berger A, Burguière AM, Cinatl J, Eickmann M, Escriou N, Grywna K, Kramme S, Manuguerra JC, Müller S, Rickerts V, Stürmer M, Vieth S, Klenk HD, Osterhaus AD, Schmitz H, Doerr HW. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med. 2003; May 15;348(20):1967-76
8. Hess M, Huggins MB, Mudzamiri R, Heincz U. Avian metapneumovirus excretion in vaccinated and non-vaccinated specified pathogen free laying chickens. Avian Pathol. 2004 Feb;33(1):35-40.
9. Bosis S, Esposito S, Niesters HG, Crovari P, Osterhaus AD, Principi N. Impact of human metapneumovirus in childhood: comparison with respiratory syncytial virus and influenza viruses. J Med Virol. 2005 Jan;75(1):101-4.
10. Mackay IM, Jacob KC, Woolhouse D, Waller K, Syrmis MW, Whiley DM, Siebert DJ, Nissen M, Sloots TP. Molecular assays for detection of human metapneumovirus. J Clin Microbiol. 2003 Jan;41(1):100-5.
11. Maertzdorf J, Wang CK, Brown JB, Quinto JD, Chu M, de Graaf M, van den Hoogen BG, Spaete R, Osterhaus AD, Fouchier RA. Real-time reverse transcriptase PCR assay for detection of human metapneumoviruses from all known genetic lineages. J Clin Microbiol. 2004 Mar;42(3):981-6.
12. Whiley DM, Syrmis MW, Mackay IM, Sloots TP. Detection of human respiratory syncytial virus in respiratory samples by LightCycler reverse transcriptase PCR. J Clin Microbiol. 2002 Dec;40(12):4418-22.
13. Rohwedder A, Keminer O, Forster J, Schneider K, Schneider E, Werchau H. Detection of respiratory syncytial virus RNA in blood of neonates by polymerase chain reaction. J Med Virol. 1998 Apr;54(4):320-7
14. Maggi F, Pifferi M, Vatteroni M, Fornai C, Tempestini E, Anzilotti S, Lanini L, Andreoli E, Ragazzo V, Pistello M, Specter S, Bendinelli M. Human metapneumovirus associated with respiratory tract infections in a 3-year study of nasal swabs from infants in Italy. J Clin Microbiol. 2003 Jul;41(7):2987-91.
15. O'Donnell DR, McGarvey MJ, Tully JM, Balfour-Lynn IM, Openshaw PJM. Respiratory syncytial virus RNA in cells from the peripheral blood during acute infection. J Pediatr. 1998 Aug;133(2):272-4.
16. Yui I, Hoshi A, Shigeta Y, Takami T, Nakayama T. Detection of human respiratory syncytial virus sequences in peripheral blood mononuclear cells. J Med Virol. 2003 Jul;70(3):481-9.
17. Xatzipsalti M, Kyrana S, Tsolia M, Psarras S, Bossios A, Laza-Stanca V, Johnston SL, Papadopoulos NG. Rhinovirus viremia in children with respiratory infections. Am J Respir Crit Care Med. 2005 Oct 15;172(8):1037-40.
18. Hall CB. The shedding and spreading of respiratory syncytial virus. Pediatr Res. 1977 Mar;11(3 Pt 2):236-9.
19. Hall CB. Respiratory syncytial virus: Its transmission in the hospital environment. Yale J Biol Med. 1982 May-Aug;55(3-4):219-23.
20. Hall CB, Douglas RG, Geiman JM. Respiratory syncytial virus infection in infants: quantitation and duration of shedding. J Pediatr. 1976 Jul;89(1):11-5.

Received: April 3, 2006 / Accepted: May 16, 2006

Address for correspondence:
Marie-Louise von Linstow
Dept. of Paediatrics 531
University of Copenhagen
Hvidovre Hospital
Kettegård Allé 30
DK - 2650 Hvidovre, Denmark
Tel.: +45 36 32 60 96
Fax: +45 36 32 37 70
E-mail: linstow@dadlnet.dk