The Burden of Human Metapneumovirus and Respiratory Syncytial Virus Infections in Hospitalized Norwegian Children

Nina Moe,[1,2] Inger Heimdal Stenseng,[1] Sidsel Krokstad,[3] Andreas Christensen,[1,3] Lars Høsøien Skanke,[1,2] Kari Ravndal Risnes,[1,2] Svein Arne Nordbø,[1,3] Henrik Døllner[1,2]

Affiliations: [1]Department of Laboratory Medicine, Children's and Women's Health, Norwegian University of Science and Technology, Trondheim, Norway, [2]Department of Pediatrics, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway and [3]Department of Medical Microbiology, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway

Correspondence: Henrik Døllner, Department of Laboratory Medicine, Children's and Women's Health, Norwegian University of Science and Health, 7006 Trondheim, Norway henrik.dollner@ntnu.no, +47-47667169 (phone), +47-72573801 (fax).

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Summary: In a 9-year long population-based Norwegian hospital study in children, HMPV appeared in epidemics and with five times lower hospitalization rate than RSV. Median HMPV shedding time was 13 days. Low levels of HMPV were rarely detected in healthy children.

ABSTRACT

Background: The burden of severe human metapneumovirus (HMPV) respiratory tract infections (RTI) in European children has not been clarified. We assessed HMPV in Norwegian children and compared hospitalization rates of HMPV and respiratory syncytial virus (RSV).

Methods: We prospectively enrolled children <16 years old hospitalized with RTI and asymptomatic controls (2006-2015). Nasopharyngeal aspirates were analyzed by polymerase chain reaction (PCR) tests for HMPV, RSV and 17 other pathogens. We genotyped HMPV-positive samples and assessed shedding time in 32 HMPV-infected children.

Results: In children with RTI, HMPV was detected in 7.3% (267/3,650) and RSV in 28.7% (1048/3,650). Among controls, 2.1% (7/339) had low HMPV levels detected by PCR, but all were culture-negative. HMPV primarily occurred from January to April and in regular epidemics. At least two HMPV subtypes occurred each season. The average annual hospitalization rates in children <5 years old with lower RTI were 1.9/1,000 (HMPV) and 10.4/1,000 (RSV). Among children with RTI, median HMPV shedding time by PCR was 13 days (range 6-28 days), but all were culture-negative (non-infectious) after 13 days.

Conclusions: HMPV appears in epidemics in Norwegian children, with a five times lower hospitalization rate than RSV. Low levels of HMPV are rarely detected in healthy children.

Keywords: burden of respiratory tract infections, hospitalization rate, human metapneumovirus, respiratory syncytial virus, healthy controls, virus shedding time.

INTRODUCTION

Human metapneumovirus (HMPV) causes upper and lower respiratory tract infections (RTI) in children, including severe diseases, such as pneumonia and bronchiolitis, in need of hospitalization [1-4]. HMPV is an epidemic virus that occurs in outbreaks all over Europe [5-9] and in other continents as well [10-14]. Aberle et al. [15] showed that in Austria the occurrence of HMPV had a biennial pattern with alternating winter and spring seasons of high activity. HMPV is included in the *Pneumoviridae* family with two main genotypes (A and B) and at least 4 subtypes (A1, A2, B1 and B2) [16-19]. Previous research has shown that HMPV genotypes A and B often circulate during the same season, while the dominant subtype may differ from one epidemic to the other [6, 7, 15, 19].

Although HMPV has been known for more than a decade, limited information exists about hospitalization rates associated with HMPV infections in European children. In three studies from the US, the average annual rates of hospitalization were reported to be from 1.0 to 1.2 per 1,000 children <5 years old, and higher rates were detected in the youngest [1, 20, 21]. Two European studies have reported somewhat higher rates [22, 23]. However, these studies had a limited duration, and there is a need for a population-based study covering a longer period from an European country.

In recent years, sensitive polymerase chain reaction (PCR) tests have been used to detect airways viruses, and it has been shown that RTI is often associated with the detection of nucleic acids from more than one virus [4, 24]. Still, viral co-detections may be common, even in asymptomatic children [25, 26]. It has been suggested that a prolonged viral shedding after an infection may be one explanation of subsequent co-detections in both asymptomatic and infected children [27-29]. Even so, a few studies with a limited number of patients found that HMPV may have a rather short excretion time [30, 31], which on the other hand could

explain why HMPV has been detected in asymptomatic controls less often than several other respiratory viruses [1, 3].

In a population based hospital study performed during a 9-year long period, we recently reported that HMPV genotypes and viral co-detections had no impacts on clinical manifestations and outcomes in HMPV-infected children [4]. Moreover, we found no differences in age-adjusted LRTI diagnoses between HMPV and RSV, while disease severity differed in relation to age: HMPV-infected children younger than 6 months old had a milder LRTI than those with RSV, whereas in children aged 12-23 months old, the opposite was observed [4].

In the present study, we aimed to assess the burden of HMPV infections in Norwegian children admitted to hospital, compared to RSV. For this purpose, we described the occurrences of HMPV, HMPV genotypes and subtypes, and RSV using the same dataset [4], and compared population-based hospitalization rates of children with LRTI due to HMPV and RSV. In addition, we wanted to evaluate HMPV in healthy children. For that reason, we assessed the occurrence of HMPV in a group of asymptomatic hospital controls, and studied the shedding time of HMPV in children with RTI.

METHODS

Study design and population

Children <16 years admitted for acute RTI with a nasopharyngeal aspirate sampled on clinical indications were prospectively enrolled at the Pediatric Emergency Department and Pediatric Department at St. Olavs Hospital, University Hospital of Trondheim, Norway, from November 2006 to July 2015 (Supplementary Figure 1, panel A). Children with cytostatic and immune-suppressive treatment were excluded. During the period from June 2007 to April 2015, similarly aged children hospitalized for elective surgery were prospectively enrolled as healthy controls (Supplementary Figure 1, panel B). None of the controls were admitted for ear, nose and throat surgery, while controls with caregiver reported symptoms of RTI during the last 2 weeks or at inclusion were excluded.

The hospital is the only hospital for children in Sør-Trøndelag County in mid-Norway, with a population of 58,443 children <16 years and 18,768 children <5 years of age (Statistics Norway). Informed written consents to participate were collected from caregivers to most of the children and from children ≥12 years during the hospital stay. Some children with RTI were enrolled after hospital discharge after passive consent. Their caregivers received written information, and the child was included if the caregivers did not resist enrollment by contacting the hospital within two weeks. In addition, we enrolled some children with acute HMPV infection, who were available for analyses of HMPV shedding time. These children were sampled during the hospitalization period and regularly after discharge during home- or outpatient visits, and until the HMPV-tests turned negative. We systematically collected baseline characteristics from a questionnaire filled out by caregivers. Clinical information was abstracted from medical records, and Regional Committees for Medical and Health Research Ethics, Central Norway, approved the study.

Clinical Classifications and Laboratory Investigations

Children admitted for acute RTI were examined and treated routinely at the discretion of medical doctors and diagnosed with upper RTI (URTI) and LRTI, as previously described [4].

Nasopharyngeal aspirates (NPA) were collected from children with RTI at admittance and during the general anesthesia in the controls. NPA were placed in a standard virus transport medium without antibiotics. Flocked swabs (Copan Italy) were used to collect follow-up nasopharyngeal samples and placed immediately into a transport medium (UTM-RT, Copan Italy). All samples were analyzed at the Department of Medical Microbiology, St. Olavs Hospital, University Hospital of Trondheim, using in-house TaqMan real-time PCR assays and conventional viral cultures for 19 respiratory pathogens, as previously described [4, 32]. Semi-quantitative results from the PCR tests were based on the cycle threshold value (Ct value), with values above 42 regarded as negative. In all 222 (83%) HMPV-positive specimens were genotyped by real-time PCR and DNA sequencing by primers targeting the F gene of HMPV [18], as previously described [4]. Some of the NPA were not typeable due to low viral loads, and others were not available. Phylogenetic comparisons of F gene sequences of 169 isolates from patients and 36 GenBank sequences representing each of the five described HMPV subtypes (A1, A2a, A2b, B1 and B2) were performed. Multiple sequences were aligned using the MUSCLE and Clustal W software. Phylogenetic analysis was inferred using the Neighbour-Joining method with evolutionary distances calculated by the Tamura-Nei method using the Geneious v.9.0.2 software.

Definitions and Statistical Analyses

A season was defined as the beginning of August to the end of July of the following year. An epidemic was the time between onset month and offset month during one season. The onset

month was the first of two consecutive months when the monthly proportion of a virus was ≥10% positive of the total number of NPA. The offset month was the last month when the monthly proportion of a virus was ≥10% positive, preceding 2 consecutive months with <10% positive samples. The peak activity month during an epidemic was the month with the highest number of children with the respective virus. Sixteen children had both HMPV and RSV in the NPA, and were included in the HMPV group.

To calculate annual hospitalization (incidence) rates we used study data, ICD-10 diagnosis statistics from the patient administrative system and population data from Statistics Norway. These data were categorized in age groups and seasons. From our study, we calculated the number of HMPV and RSV-positive children with LRTI diagnosis staying ≥24 hours. Twelve children with LRTI had both HMPV and RSV, and were included in the HMPV group. These ICD-10 codes were included: pneumonia J10.0, J11.0, J12.0-J12.9, J13-J15, bronchitis J20, bronchiolitis J21, unspecified LRTI J22 and asthma exacerbation J45-46.

The duration of HMPV shedding was estimated by Kaplan-Meier analysis in 32 available children. In total, 93 respiratory specimens, in average 3 per child, were collected at a median 4.0, 8.5 and 13.0 days after symptom onset. Four HMPV-positive specimens in the last sampling were censored. Samples with Ct values >42 were encoded with a Ct value ≥42.1 for the HMPV shedding analysis.

We used the χ^2-test or Fischer's Exact Test, Student t-test, Mann-Whitney U-test or Kruskal-Wallis test to compare categorical, parametric and non-parametric variables, as appropriate. Repeated measures were analyzed by Friedman test for ordinal variables and Cochran's Q test for dichotomous variables. *P*-values < .05 (two-sided) were considered statistically significant and the data was analyzed using IBM SPSS Statistics 22 and SigmaPlot 13.0.

RESULTS

HMPV and RSV among children with RTI and asymptomatic controls

Among 3650 children admitted with RTI, HMPV was detected in 7.3% (267/3,650), RSV in 28.7% (1048/3,650) and 64.0% had other viruses or were virus-negative (Supplementary Figure 1, panel A). Infected children with HMPV and RSV had a median age of 17.7 months (IQR 9.1-29.7) and 7.4 months (IQR 2.5-17.7) ($P < .001$), respectively. Baseline and clinical characteristics are presented in Table 1. Three children were hospitalized twice with HMPV infection within a 5-year period, elicited by unknown or different subtypes. Among the asymptomatic controls with a median age of 39.4 months (IQR 21.0-63.3), HMPV was detected in 2.1% (7/339) and RSV in 3.2% (11/339) (Supplementary Figure 1, panel B). HMPV and RSV more frequently were detected among children with RTI than among controls (both $P < .001$). The median Ct value of HMPV among children with RTI (28.0, IQR 24.2-32.1) was lower than among controls (38.9, IQR 37.6-39.2) ($P < .001$). In all 43.8% (117/267) of infected children were HMPV culture-positive at admittance compared to none of the controls (0/7). Similarly, the median Ct value of RSV among children with RTI (23.5, IQR 20.9-26.8) was lower than among controls (30.9, IQR 30.3-33.2) ($P < .001$), and 91.4% (958/1048) and 54.5% (6/11) respectively, were RSV culture-positive in the same two groups.

Seasonal trends and epidemics

The detection of HMPV varied from 2.6% to 12.4% of the children in each of 9 seasons, an average of 7.3% per season (Supplementary Figure 2). RSV was more frequent than HMPV, and varied from 21.3% to 39.0%, an average of 28.7% per season. Analyses of the monthly HMPV-distribution during all nine years showed that HMPV mostly appeared from January to April (74.2%, 198/267). Going more into detail, HMPV appeared from January-March in 62.5%, April-June in 23.2%, October-December in 13.1% and July-September in 1.1%.

Furthermore, the occurrence of HMPV in the period from January to March in odd and even years (even year, i.e. 2006/07) was equal (P = .730) (Supplementary Figure 3). RSV was particularly frequent from January to March (71.2%, 746/1,048). Looking on epidemics, HMPV appeared from October to July in 2 to 6 consecutive months, with a median outbreak duration of 3.5 months (Supplementary Figure 2). Four seasons had peak activity in January and February, while the other four seasons had peak activity in March or later. The winter HMPV epidemics had higher peaks (winter: 11-20 HMPV-positives per month vs spring-summer: 3-8 HMPV-positives per month) and a longer duration (winter: median 5 months vs spring-summer: 2.5 months) than the spring-summer HMPV epidemics (P = .004 and P = .057, respectively). RSV-epidemics occurred in all 9 seasons and had a median duration of 5 months, varying from 5 to 8 months from October to July. RSV epidemics had a longer median duration than HMPV epidemics (P = .011). Additionally, HMPV epidemics appeared before, during or after RSV epidemics.

HMPV genotypes and subtypes

Genotype B was detected in 56.8% (126/222) and genotype A in 43.2% (96/222). HMPV A and B co-circulated each season, although the distributions of each genotype changed during the seasons (P <.001) (Figure 1 and Supplementary Table 1). Among the HMPV genotype B positive samples, 37 were subtype B1 and 89 were subtype B2. In genotype A, 12 samples were subtype A2a, 80 were subtype A2b and 4 were subtype A2 (unassigned), while no samples were positive for subtype A1. Two or more subtypes were detected every season, and one or two subtypes dominated in each season. Phylogenetic analyses of the F gene region showed that several strains circulated each year. No clusters or new strains were detected during the 9 year-long study period (Supplementary Figure 4).

Hospitalizations rates of LRTI during 9seasons

Altogether, 1130 children were hospitalized with LRTI with either HMPV (n = 186) or RSV (n = 944). The mean annual hospitalization rate of HMPV-associated LRTI in children <5 years was 1. 9/1,000 children (Table 2). The youngest children aged 0-11 months old had a rate of 3.1/1,000 children, and 12-23 months old had a rate of 3.4/1,000 children. Children with RSV had higher hospitalization rates than HMPV: 10.4/1,000 children <5 years, 27.5/1,000 children aged 0-11 months and 14.7/1,000 children aged 12-23 months. In children ≥24 months, the rates gradually decreased in both HMPV- and RSV-infected children with increasing age.

Shedding of HMPV

Among all HMPV-positive inpatients, 32 were available for the shedding analyses. They had a median age of 16.0 months (IQR 7.5-26.8), 30 out of 32 had LRTI and 2 out of 32 had URTI (Supplementary Table 2). A Kaplan-Meier analysis estimated that 50% (median) and 100% of 32 children were virus PCR-negative after 13.0 (95% CI 11.5-14.5) and 28.0 days, respectively, from the onset of symptoms (Figure 2), with the shedding time varying from 6.0-28.0 days. The NPA taken at admittance had a median Ct value of 23.8 and 84.4% (27/32) were culture-positive (Supplementary Table 2). The first follow-up samples had a median Ct value of 34.7, and only 15.6% (5/32) were still culture-positive. The second follow-up samples had a median Ct value ≥42.1, the value encoded for virus-negatives, and none out of 20 samples were culture- positive. The median Ct values gradually increased, and the rate of culture-positive samples gradually decreased from admittance to first and second follow-up samples (both $P < .001$), and all children gradually improved.

DISCUSSION

The present data from our population-based study performed during nearly 9 years show that HMPV is associated with a substantial disease burden, and annually causes an average of 1. 9 hospitalizations per 1,000 Norwegian children younger than 5 years, although HMPV is still associated with a five times lower hospitalization rate than RSV. Several findings have confirmed that HMPV is an epidemic virus: First, HMPV occurred in regular winter and spring-summer outbreaks during the entire study period. Secondly, the infected children initially had high viral levels, but a short viral shedding time, and thirdly, no asymptomatic controls had a HMPV-positive culture, although a few had low levels of HMPV as detected by PCR.

On average, HMPV was detected in 7.3% of all children admitted with RTI during the whole period, but it varied considerably from only 2.6% to 12.4% per season. Most previous studies from countries in the Northern hemisphere measured the occurrence over shorter periods, but found relative similar figures and seasonal variations [1, 3, 8, 13-15]. HMPV appeared mostly from January to April and regularly caused outbreaks of a median of 5 months' duration, peaking in the winter months. Smaller outbreaks with a median duration of 2.5 months appeared during the spring and early summer months, and coincided with a reduction in the total number of children admitted with RTI. In addition, the occurrence of HMPV from January to March was quite similar in both odd and even years, in contrast to observations from southern Europe, with alternating epidemics in winter and spring-summer every other year [15, 33]. We speculate as to whether this may be related to the cold climate in our country compared to the warmer climate in the southern part of Europe [34]. RSV outbreaks occurred in every season and lasted an average of 5 months, and most often peaked in January to March. As previously described, HMPV outbreaks appeared before, overlapping with or after RSV [5].

We detected all known HMPV subtypes, except for subtype A1, with subtype B2 being the most frequent over the entire period. In line with other studies [6, 7, 15, 19], the distribution of subtypes showed great seasonal variation. In every season one or two subtypes dominated, and at least two subtypes circulated, but no new strains or clusters were detected. We previously have reported that HMPV genotypes and subtypes were associated with very similar clinical manifestations [4].

In the present study, the average annual hospitalization rate of HMPV-related LRTI over 9 seasons was 1.9/1,000 children aged <5 years old. Children in the youngest age groups had higher rates. We used a strict definition of severe HMPV infection including only children with a hospital stay ≥24 hours and LRTI, which might explain why our estimates differ from three US studies that included a broader spectrum of respiratory infections, and reported estimates from 1.0- to 1.2/1,000 children <5 years old [1, 20, 21]. Two European studies reported HMPV-related hospitalizations rates comparable with ours. A study from Spain [23], based on 3 seasons, reported that 2.6/1,000 children <3 years old were hospitalized, and in a single season study from UK [22] the rate was reported to be 1.3/1,000 children <6 years old. Our finding of higher hospitalization rate in 12-23 months-old children differ with the findings in all previous studies [1, 20-23], and may also relate to our strict inclusion criteria. The hospitalization rates of children with RSV-related LRTI in our study were in line with findings from previous Norwegian [35], European [36, 37] and American studies [38, 39], thereby confirming that HMPV causes hospitalization less often than RSV in Europe and US.

To test the hypothesis that low detection rates and low levels of HMPV in healthy children may be a result of virus shedding after previous RTI, we first measured the rate of HMPV-positive samples among a group of asymptomatic children. A few percent had a positive PCR test with high Ct levels, thus corresponding to low viral loads, but all were

virus-negative by culture. We also studied a group of children with HMPV infection with repeated specimens sampled, who had low Ct values (high viral loads) and a high rate of positive cultures initially. During the progress of the disease, these children improved clinically, viral loads gradually decreased and all became virus-negative by culture after 13 days. Despite these changes, half of the children were still virus-positive by PCR test after 13 days and all were negative after 28 days only. Taken together, our observations along with observations done by others [1, 30, 31, 40, 41], support that a positive PCR test for HMPV in healthy children is unlikely to indicate an asymptomatic infection, and we speculate whether it instead indicates the presence of small amounts of viral nucleic acids after a previous HMPV infection. Others [40, 42] have demonstrated a 2-3-week-long shedding time in children with RSV infection, which in a similar way may explain the low detection rate of RSV at low viral levels in the controls of the present study.

As indicated by the hospitalization rates, the incidence of severe HMPV infection, decreased by age. In addition, only 1% of previously healthy children were admitted with recurrent HMPV infections elicited by unknown or different HMPV subtypes. Previous research has shown that most children become seropositive during the first 5 years of life [43], while data from experimental studies suggest that certain HMPV subtypes may not stimulate an adequate immune response in all cell types [44]. However, our clinical data support that healthy children usually develop a robust immunity against most HMPV subtypes during childhood. On the other hand, outside a hospital setting, others have shown that HMPV may still cause recurrent mild RTI in children [45] and adults [46]. Moreover, children [47] and adults [48] with impaired immunity may be prone to severe HMPV infections, even with a high seroprevalence at all ages [49].

It is a strength of the present population-based study, that we prospectively enrolled children at all ages from the same county in mid-Norway, and to the only existing pediatric

hospital in this region during a long period. It is also an advantage that we used the same PCR tests and viral cultivation methods during the entire period. However, the controls were sampled during anesthesia and we have not adjusted for the fact that controls were in general older than children with RTI. Moreover, controls were not contacted after sampling to assess whether subsequent RTI symptoms had occurred. All factors might have contributed to higher viral detection rates among controls. Some HMPV-positive samples were not genotyped and a few were unassigned A2. Hence, the A1 subtype might have been present, and the pattern of circulating HMPV subtypes might have been even more heterogenic than described.

In conclusion, HMPV occurs in winter and spring-summer epidemics in Norwegian children, but the hospitalization rate is 5 times lower than RSV. All known HMPV subtypes, except for A1, circulate in Norway. Children are rarely hospitalized twice with HMPV infection. Children have a short HMPV shedding time and may not be infectious for more than 13 days, and the short shedding time may also explain the low HMPV detection rate among asymptomatic children.

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FIGURE LEGENDS

Figure 1. Distribution of human metapneumovirus (HMPV) and HMPV subtypes during 9 seasons.

Number of HMPV positive samples on the Y-axis. Tot HMPV (black solid line) indicates the total number of HMPV-positive samples including samples with known and unknown subtypes.

*Unassigned A2.

Figure 2. Kaplan-Meier analysis of human metapneumovirus (HMPV) shedding time in children with respiratory tract infection.

Y-axis represents estimated proportion of HMPV-positive nasopharyngeal samples and X-axis represents number of days from onset of symptoms until HMPV-negative sample. The estimated proportion (solid line) is presented with the 95% confidence interval (stippled lines).

Notes

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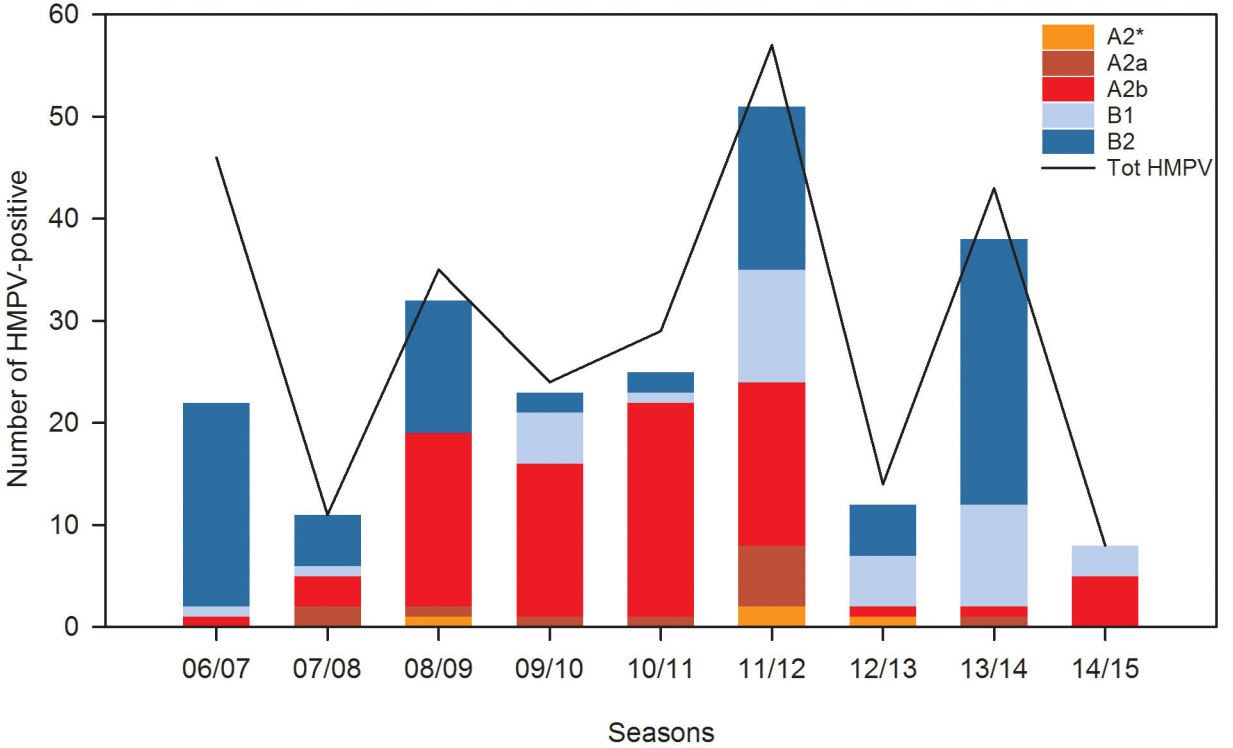
Presentations of data at meetings: The data has not been presented at international meetings yet.

Correspondence: Henrik Døllner, Department of Laboratory Medicine, Children's and Women's Health, Norwegian University of Science and Technology, 7006 Trondheim, Norway, henrik.dollner@ntnu.no, +47 47667169 (phone), +47-72573801 (fax). Second corresponding author Nina Moe, Department of Laboratory Medicine, Children's and Women's Health, Norwegian University of Science and Technology, 7006 Trondheim, Norway, nina.moe@ntnu.no, +47 72574046 (phone), +47-72573801 (fax).


60
50
40
30
20
10
0
Number of HMPV-positive
A2*
A2a
A2b
B1
B2
Tot HMPV
06/07
07/08
08/09
09/10
10/11
11/12
12/13
13/14
14/15
Seasons

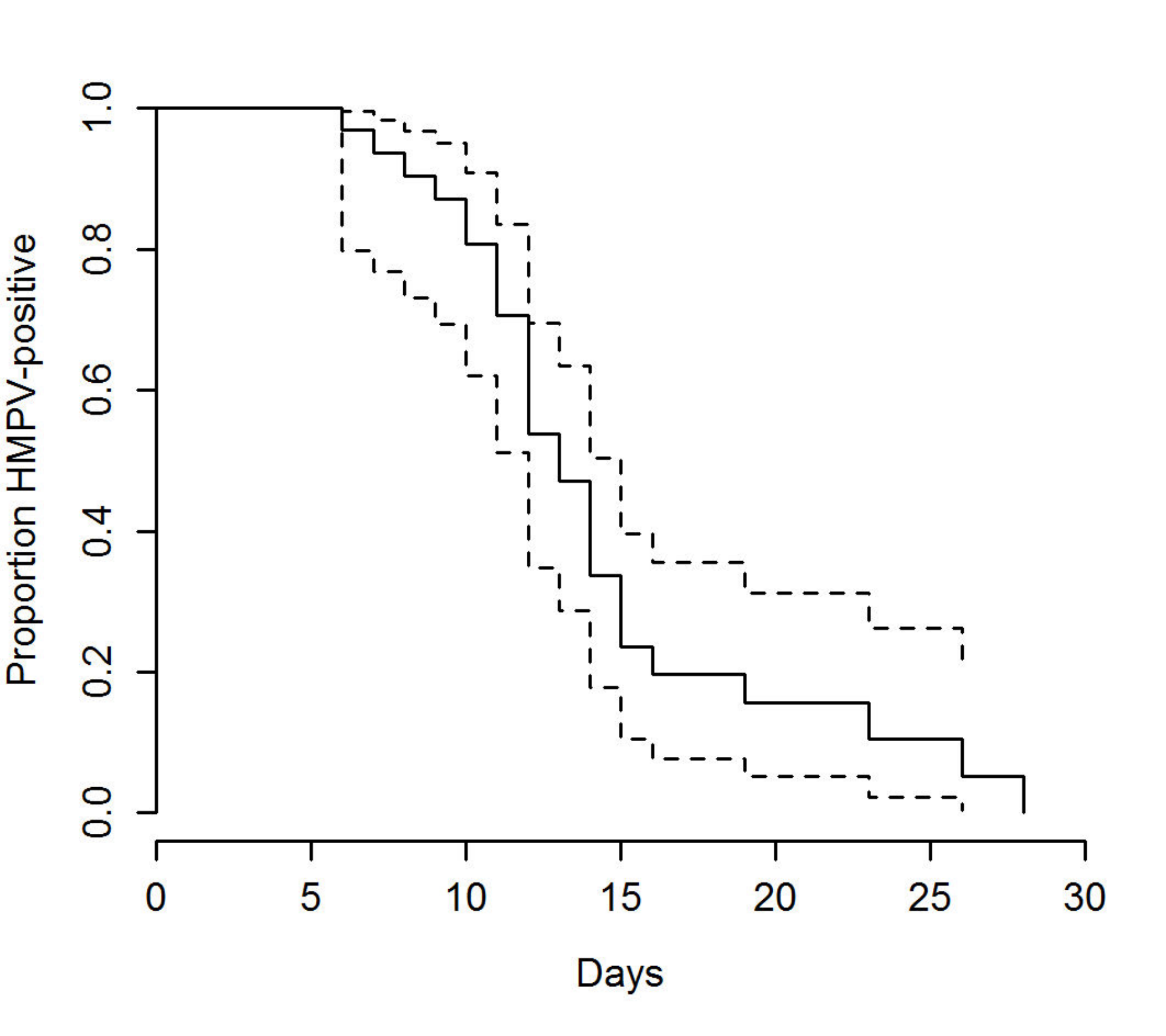

Proportion HMPV-positive
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
30
Days

Table 1. Baseline and Clinical Characteristics of Children with Respiratory Tract Infections Due to HMPV and RSV

Characteristic	HMPV[a] (n = 267)	RSV (n = 1048)
Age (median, IQR)	17.7 (9.1-29.7)	7.4 (2.5-17.7)
Age group, months		
<6	41 (15.4)	462 (44.1)
6-11	46 (17.2)	187 (17.8)
12-23	89 (33.3)	256 (24.4)
24-59	75 (28.1)	126 (12.0)
≥60	16 (6.0)	17 (1.6)
Gender (male)	154 (57.7)	603 (57.5)
Premature born (gestational age <36 weeks)	51 (19.1)	147 (14.0)
≥1 chronic disease	89 (33.3)	187 (17.8)
Upper respiratory tract infection	33 (12.4)	42 (4.0)
Lower respiratory tract infection	234 (87.6)	1006 (96.0)
Bronchiolitis	89 (33.3)	657 (62.7)
Pneumonia	84 (31.5)	201 (19.2)
Asthma exacerbation	35 (13.1)	107 (10.2)
Obstructive bronchitis	11 (4.1)	31 (3.0)
Unspecified	15 (5.6)	9 (0.9)
Outpatients (hospital stay <24 hours)	64 (24.0)	69 (6.6)
Inpatients (hospital stay ≥24 hours)	203 (76.0)	979 (93.4)
Upper respiratory tract infection	17 (8.4)	35 (3.6)
Lower respiratory tract infection	186 (91.6)	944 (96.4)

Length of stay (median, IQR)	4.0 (2.0-6.0)	4.0 (2.0-6.0)

Data are presented as absolute numbers and percent in brackets, except from age and length of stay in median and interquartile range (IQR).

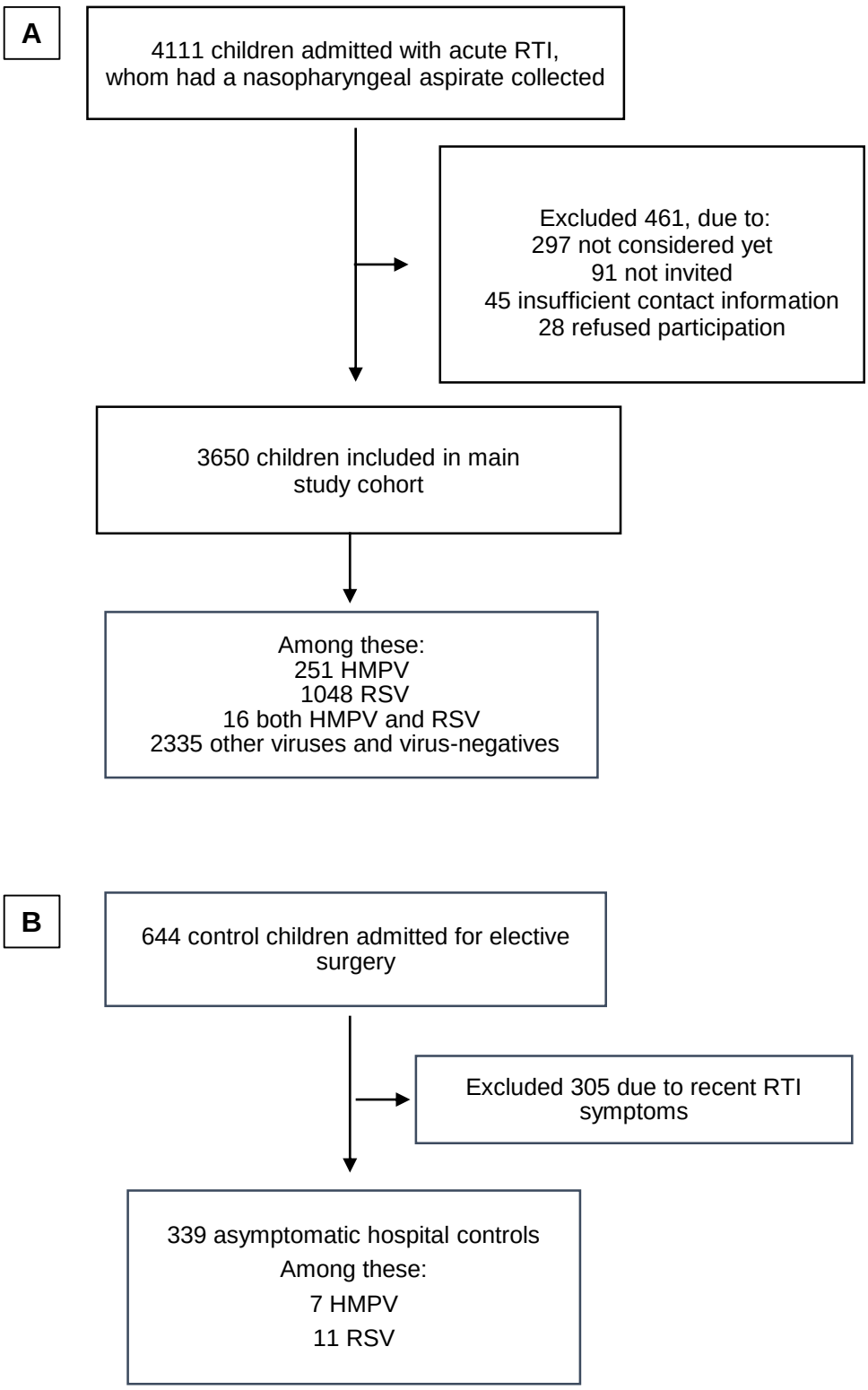
Abbrevations: HMPV, Human Metapneumovirus; RSV, Respiratory Syncytial Virus.

[a]Sixteen children had both HMPV and RSV and were included in the HMPV group only.

Table 2. Incidence Rates of Hospitalization per 1000 children with Lower Respiratory Tract Infection, by Virus (HMPV or RSV), Season and Age

Season	Age 0-11 months		Age 12-23 months		Age 24-59 months		Age 5-16 years		Age 0-59 months	
	HMPV	RSV	HMPV	RSV	HMPV	RSV	HMPV	RSV	HMPV	RSV
2006/07	5.9	24.9	4.3	17.9	1.8	2.2	0.2	0.2	3.2	10.4
2007/08	0.5	35.2	2.4	8.9	0.0	3.3	0.0	0.0	0.5	11.6
2008/09	4.0	19.7	5.0	13.4	1.2	1.5	0.1	0.1	2.5	8.3
2009/10	3.4	25.2	1.0	13.6	1.2	2.5	0.0	0.0	1.6	9.5
2010/11	2.4	31.8	2.5	12.9	0.6	3.7	0.0	0.1	1.3	12.1
2011/12	5.2	18.2	6.9	12.6	2.1	1.3	0.1	0.0	3.7	7.3
2012/13	1.5	40.7	1.3	19.4	0.5	2.9	0.0	0.1	0.8	14.1
2013/14	2.7	18.2	6.4	10.1	1.2	1.5	0.1	0.0	2.4	6.6
2014/15	2.5	33.3	1.0	23.1	1.0	2.1	0.0	0.4	1.3	13.4
Mean	3.1	27.5	3.4	14.7	1.1	2.3	0.06	0.1	1.9	10.4
95% CI	2.0-4.2	22.1-32.9	1.9-4.9	11.7-17.7	0.7-1.5	1.8-2.8	0.01-0.11	0.03-0.17	1.2-2.6	8.6-12.2

Abbreviations: HMPV, Human Metapneumovirus; RSV, Respiratory Syncytial virus; CI, Confidence Interval.

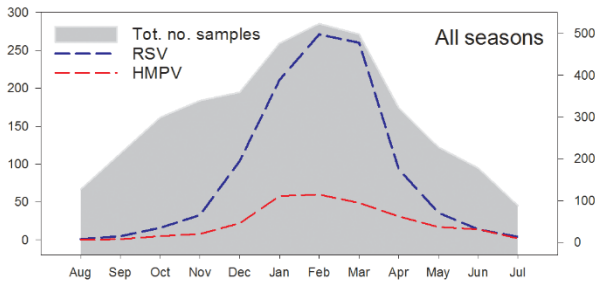


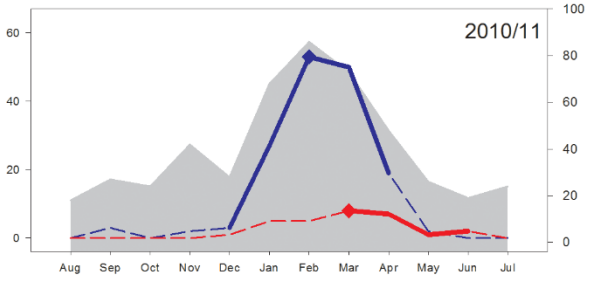
Supplementary Figure 1. Study flow chart, where (A) represents children admitted with acute respiratory tract infections from November 2006 to July 2015 and (B) represents hospital controls admitted for elective surgery from June 2007 to April 2015. HMPV indicates human metapneumovirus and RSV indicates respiratory syncytial virus.

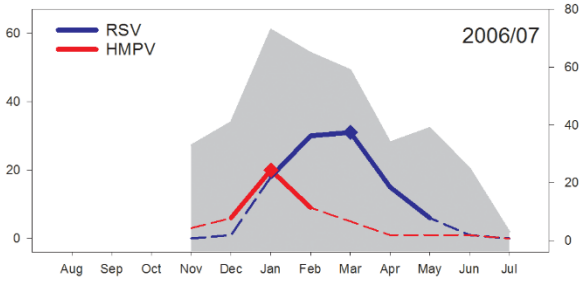
Supplementary Figure 2. Detection of HMPV and RSV among children with respiratory tract infection according to month and season.

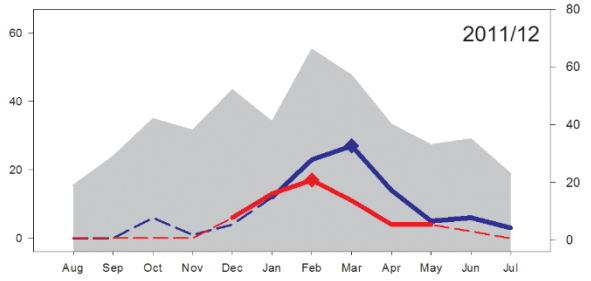
Gray shade represents the total number of samples tested, with numbers at the right Y-axis. Dashed lines indicate the detection of HMPV (red) and RSV (blue) and solid lines indicates HMPV epidemic (≥10% positive) (red) and RSV epidemic (≥10% positive) (blue), with numbers at the left Y-axis. Diamonds are peak activity month during HMPV epidemics (red) and RSV epidemics (blue). During the 2007/08 season, no HMPV epidemic occurred and therefore no peak activity month is marked. The peak activity month during an epidemic was the month with highest number of children with the respective virus.

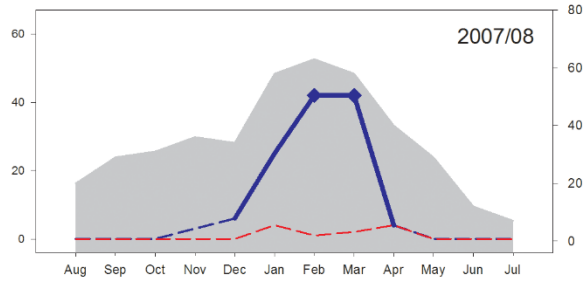
HMPV indicates human metapneumovirus and RSV indicates respiratory syncytial virus.

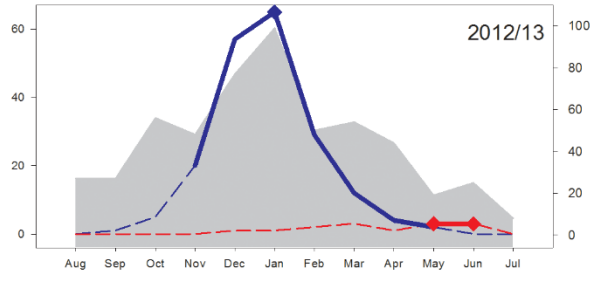

Tot. no. samples
RSV
HMPV
All seasons
Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul

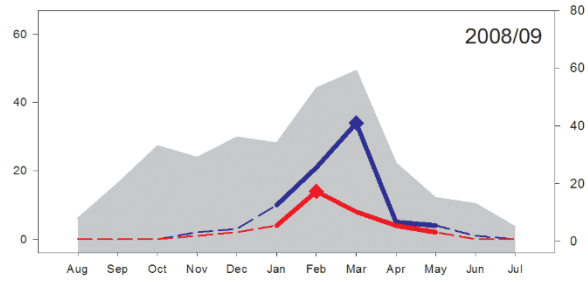

2010/11
Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul

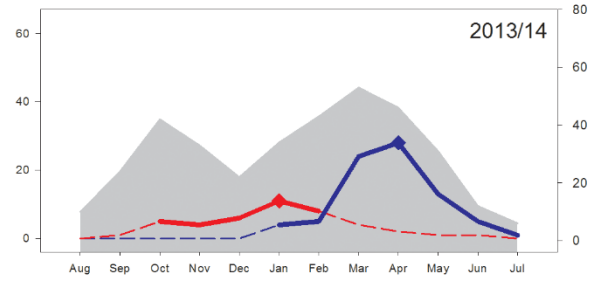

RSV
HMPV
2006/07
Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul

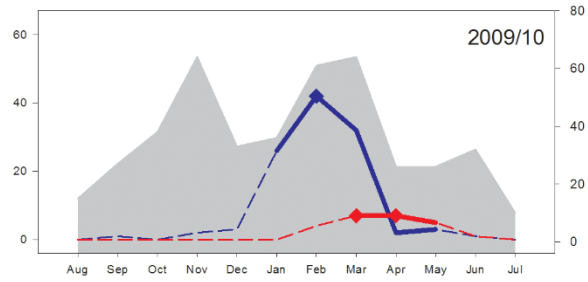

2011/12
Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul

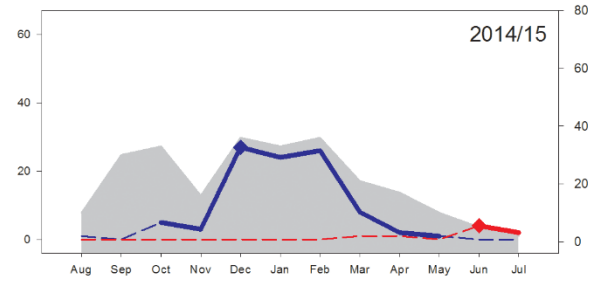

2007/08
Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul

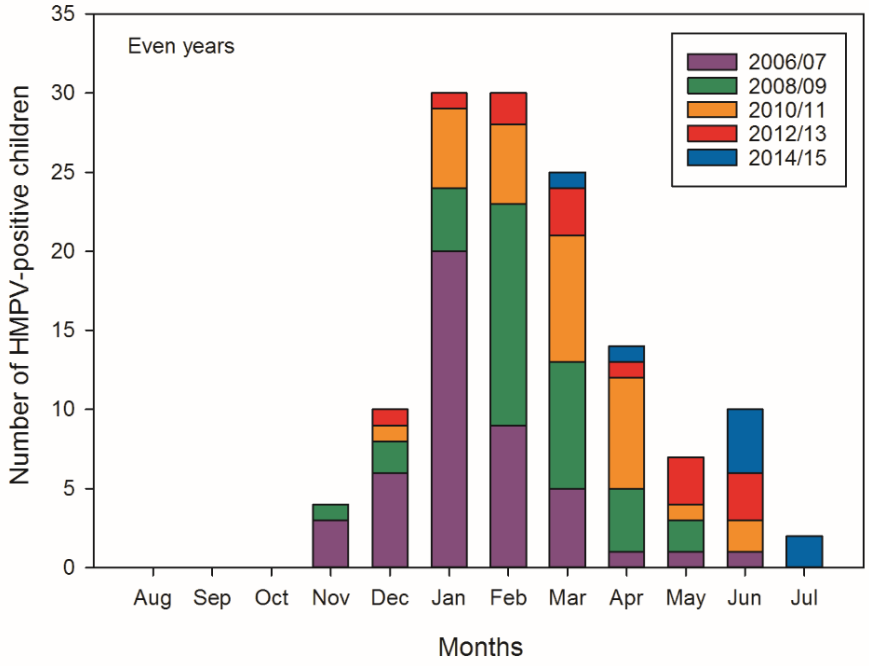

2012/13
Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul

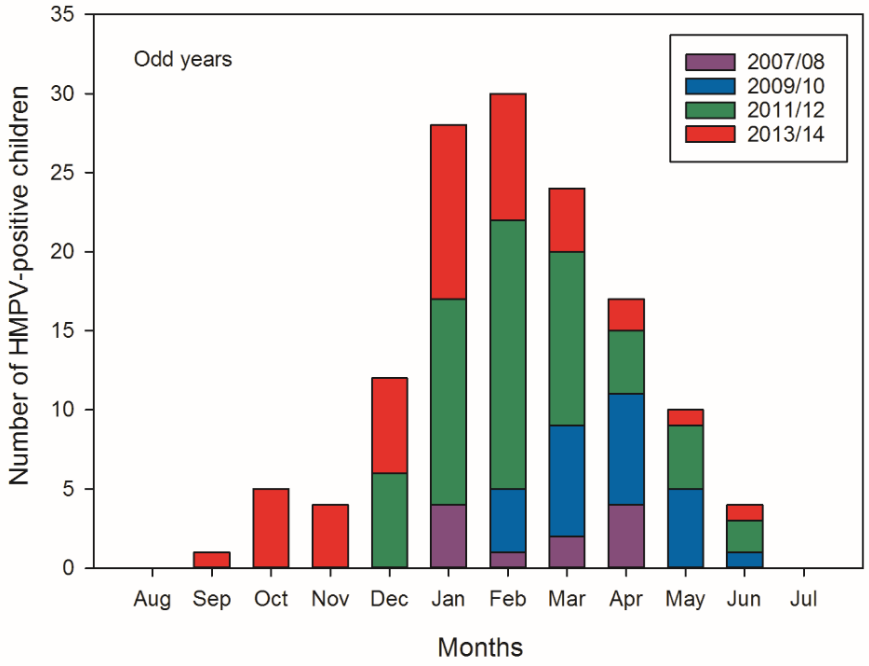

2008/09
Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul


2013/14
Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul

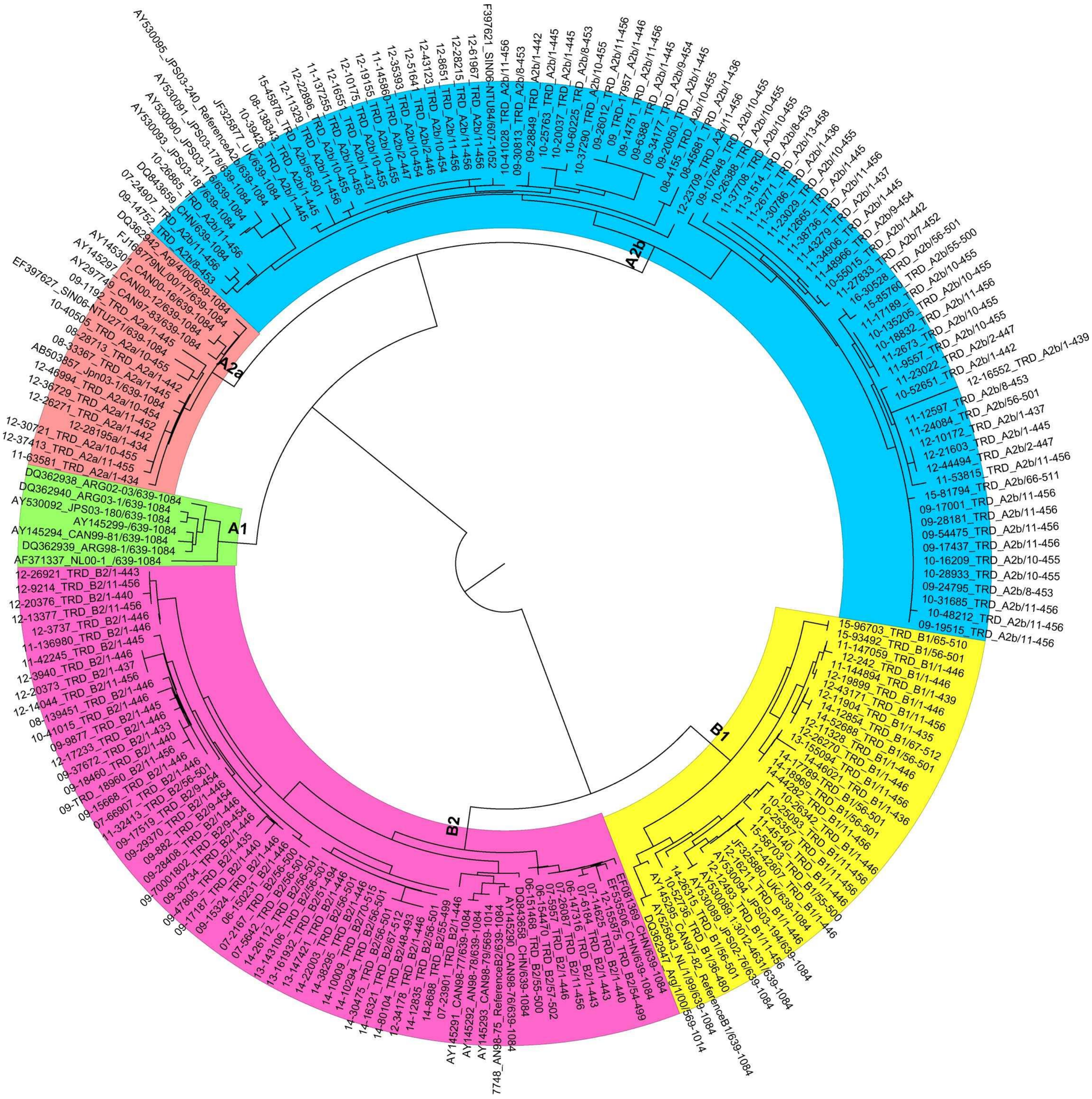

2009/10
Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul


2014/15
Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul





Supplementary Figure 3. Occurrence of human metapneumovirus (HMPV) in even and odd years.
Even years, i.e. 2006/07.



Supplementary Figure 4.
Phylogeny of 169 patient sequences obtained by partial sequencing of the HMPV F gene and 36 GenBank sequences. Phylogenetic analysis was constructed by the Neighbour-Joining method with evolutionary distances calculated by the Tamura-Nei method using the Geneious v.9.0.2 software. The sequences from this study are labelled by year of sample collection, specimen identifier and TRD (Trondheim). The GenBank strains are labelled with accession number and geographic origin. ARG, Argentina; AUS, Australia; CAN, Canada; CHN, China; JPS, Japan; NL, Netherlands; SIN, Singapore; UK, United Kingdom. The figure is produced using the FigTree version 1.4.3 program.

Supplementary Table 1. Circulation of HMPV During Nine Seasons, According to Subtypes and in Total

Season	A2a	A2b	A2[a]	B1	B2	Unknown	Total HMPV
2006/07	0 (0.0)	1 (2.2)	0 (0.0)	1 (2.2)	20 (43.5)	24 (52.2)	46 (100.0)
2007/08	2 (18.2)	3 (27.3)	0 (0.0)	1 (9.1)	5 (45.5)	0 (0.0)	11 (100.0)
2008/09	1 (2.9)	17 (48.6)	1 (2.9)	0 (0.0)	13 (37.1)	3 (8.6)	35 (100.0)
2009/10	1 (4.2)	15 (62.4)	0 (0.0)	5 (20.8)	2 (8.3)	1 (4.2)	24 (100.0)
2010/11	1 (3.4)	21 (72.4)	0 (0.0)	1 (3.4)	2 (6.9)	4 (13.8)	29 (100.0)
2011/12	6 (10.5)	16 (28.1)	2 (3.5)	11 (19.3)	16 (28.1)	6 (10.5)	57 (100.0)
2012/13	0 (0.0)	1 (7.1)	1 (7.1)	5 (35.7)	5 (35.7)	2 (14.3)	14 (100.0)
2013/14	1 (2.3)	1 (2.3)	0 (0.0)	10 (23.3)	26 (60.5)	5 (11.6)	43 (100.0)
2014/15	0 (0.0)	5 (62.5)	0 (0.0)	3 (37.5)	0 (0.0)	0 (0.0)	8 (100.0)
Total	12 (4.5)	80 (30.0)	4 (1.5)	37 (13.9)	89 (33.3)	45 (16.9)	267 (100.0)

Data presented as absolute number and percent in parenthesis out of total each season and in total out of all seasons.

[a]Unassigned.

HMPV indicates human metapneumovirus.

Supplementary Table 2. HMPV Shedding in Children with Respiratory Tract Infection, with Viral Loads, Culture Results and Duration of Symptoms at Three Sampling Times

Child no.	Age, mo	Diagnosis	Sampling at admittance			First follow-up sampling			Second follow-up sampling		
			Days[a]	Ct[b]	Culture[c]	Days	Ct	Culture	Days	Ct	Culture
1	18.5	bronchiolitis	2	19.2	Pos.	4	23.6	Neg.	11	≥42.1[d]	Neg.
2	91.4	pneumonia	6	22.2	Pos.	12	≥42.1	Neg.			
3	18.5	pneumonia	7	29.1	Pos.	9	38.6	Neg.	14	≥42.1	Neg.
4	23.3	bronchiolitis	5	33.1	Neg.	9	≥42.1	Neg.			
5	24.9	pneumonia	6	28.8	Pos.	7	29.5	Neg.	11	≥42.1	Neg.
6	19.8	pneumonia	3	23.9	Pos.	6	22.7	Pos.	10	37.9	Neg.
7	12.0	pneumonia	4	24.9	Pos.	6	27.4	Neg.			
8	9.1	bronchiolitis	5	21.3	Pos.	10	33.9	Neg.	16	≥42.1	Neg.
9	19.2	pneumonia	2	21.4	Pos.	6	31.1	Neg.	11	≥42.1	Neg.
10	12.6	pneumonia	6	30.5	Pos.	10	33.3	Neg.	15	≥42.1	Neg.
11	27.4	unspec. LRTI[e]	3	25.1	Pos.	10	≥42.1	Neg.			
12	15.5	URTI[f]	2	23.6	Pos.	7	29.9	Pos.	10	32.0	Neg.
13	1.8	bronchiolitis	2	22.9	Pos.	6	34.2	Pos.	12	≥42.1	Neg.

14	1.6	bronchiolitis	5	31.5	Neg.	12	≥42.1	Neg.			
15	32.7	pneumonia	6	20.5	Pos.	17	27.9	Neg.	22	30.4	Neg.
16	3.1	bronchiolitis	2	21.7	Pos.	4	25.5	Pos.	6	30.8	Neg.
17	12.3	bronchiolitis	3	28.8	Neg.	7	32.4	Neg.	10	38.0	Neg.
18	18.1	URTI	4	27.2	Pos.	12	≥42.1	Neg.			
19	16.4	pneumonia	4	27.4	Pos.	8	≥42.1	Neg.			
20	56.6	pneumonia	3	19.3	Pos.	7	36.2	Neg.	11	36.7	Neg.
21	174.2	asthma exac.	5	33.7	Neg.	9	38.2	Neg.	14	≥42.1	Neg.
22	60.1	pneumonia	2	20.7	Pos.	7	31.9	Neg.	14	≥42.1	Neg.
23	31.4	asthma exac.	4	30.9	Neg.	6	≥42.1	Neg.			
24	14.8	pneumonia	5	22.2	Pos.	12	30.7	Neg.	17	37.4	Neg.
25	11.5	pneumonia	5	24.5	Pos.	10	35.2	Neg.	15	≥42.1	Neg.
26	6.1	bronchiolitis	2	23.3	Pos.	6	26.2	Neg.	9	35.4	Neg.
27	33.6	pneumonia	5	28.3	Pos.	10	≥42.1	Neg.			
28	1.7	bronchiolitis	4	21.2	Pos.	13	≥42.1	Neg.			
29	3.5	bronchiolitis	5	20.2	Pos.	8	29.0	Pos.	15	≥42.1	Neg.
30	13.5	asthma exac.	5	21.5	Pos.	14	36.6	Neg.	20	33.8	Neg.
31	7.0	asthma exac.	5	26.1	Pos.	19	≥42.1	Neg.			
32	0.5	bronchiolitis	2	23.8	Pos.	7	≥42.1	Neg.			

Median	16.0			4.0	23.8			8.5	34.7			13.0	≥42.1	
Pos. cultures, n (%)						27 (84.4)				5 (15.6)				0 (0.0)

Abbreviations; Ct value, cycle threshold value; HMPV, human metapneumovirus.

[a]Days with symptoms from onset of respiratory tract infection to sampling.

[b]Ct value in respiratory samples at sampling.

[c]Viral culture positive or negative for HMPV at sampling.

[d]Virus negative was encoded with a Ct value of ≥42.1

[e]Unspecified lower respiratory tract infection.

[f]Upper respiratory tract infection.