MAJOR ARTICLE

Rhinovirus Transmission within Families with Children: Incidence of Symptomatic and Asymptomatic Infections

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Background. Rhinoviruses are the most common cause of respiratory tract infections, but the transmission in families has not been studied using sensitive and specific molecular detection methods.

Methods. Children hospitalized for any infection were screened for rhinoviruses. Eight families with a rhinovirus-positive index child and 16 families with a rhinovirus-negative index child were monitored for 3 weeks for disease symptoms, and the presence and quantity of rhinoviruses in nasal swab samples were determined by quantitative reverse transcription–polymerase chain reaction. Rhinoviruses were further identified by melting temperature and partial sequence analysis.

Results. The rates of rhinovirus infection were 1.00 cases per person among the 17 siblings and 0.50 cases per person among the 14 parents of rhinovirus-positive index patients; the rates were 0.54 cases per person among the 24 siblings and 0.23 cases per person among the 30 parents of rhinovirus-negative index patients. Symptomatic infections were associated with an age of <7 years but not with a high copy number of rhinovirus genomes. Virus typing revealed the transmission routes of the viruses and showed that several virus types could circulate in the families simultaneously.

Conclusions. Rhinoviruses are frequently transmitted from children to other family members. Most rhinovirus infections in young children are symptomatic, but secondary infections in adults are often asymptomatic. Multiple virus types circulate simultaneously in families.

Rhinoviruses are the most common cause of respiratory tract infections in individuals of all ages, causing one-half of common colds annually and 90% of colds during the autumn epidemic season in adults [1–4]. Otitis media is the most frequent complication of rhinovirus infection in children; in a study of children <2 years of age, rhinovirus was detected in 40% of acute otitis media episodes [5]. Rhinoviruses are major causes of expiratory wheezing in children. Rhinoviral wheezing illnesses often necessitate hospital admission [6–9] and may develop into asthma [10, 11]. In concert with bacteria, rhinoviruses are probably important agents in the development of pneumonia [12, 13]. The economic significance of rhinovirus infection at the community level is substantial [14, 15].

Knowledge of the transmissibility of rhinoviruses is the basis for intervention studies targeting the spread of infection in families, day care centers, and schools. Earlier studies of rhinovirus epidemiology and transmission were mainly based on viral cultures. These studies showed that several rhinovirus serotypes circulate in the population at the same time, whereas the transmission rates of natural rhinovirus infection within families were rather low (11%–56%) [1, 16–20]. Experimentally induced rhinovirus infection in human volunteers can be transmitted by both the aerosol route and hand contact, but transmission efficiency is, however, low and requires close contacts between virus donors and recipients [21–24]. More recently, rapid and sensitive reverse transcription–polymerase chain reaction (RT-PCR) methods have largely replaced culture in the detection of rhino-

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viruses. In a study of adults with common cold, 121 nasopharyngeal samples were positive for rhinoviruses by both culture and RT-PCR, 65 samples were positive by RT-PCR only, and 6 samples were positive by culture only [25]. RT-PCR can be expected to give a more detailed view than culture of the transmission of rhinoviruses within families, and it permits quantification of viral genomes in samples, as well as molecular identification of virus types. Differentiation between simultaneously circulating virus strains is needed to determine transmission chains.

Of interest, rhinoviruses have been detected by RT-PCR in 15%–30% of asymptomatic individuals [26–29], but the clinical significance of these findings is presently unknown. They may reflect persistent asymptomatic infection, the asymptomatic period before the development of symptomatic infection, or carriage of the virus after symptomatic infection. Differences in the replication efficiency of individual virus types and in the ability of the immune system to prevent clinical infection may play a role here. The role of asymptomatic virus-positive individuals as reservoirs in the transmission of rhinoviruses is unknown. Moreover, the high frequency of positive RT-PCR findings in asymptomatic individuals makes it difficult to ascertain the causative role of rhinoviruses detected by RT-PCR in patients with respiratory symptoms. Viral load could be hypothesized to be a parameter that would differentiate between symptomatic respiratory infections and carrier state.

The aims of our study were to analyze the transmission of rhinovirus infection in families, using quantitative RT-PCR combined with melting temperature and sequence analysis for virus detection and typing, and to compare the incidence, sources, and transmissibility of asymptomatic infections with those of symptomatic infections.

MATERIALS AND METHODS

Study description. We obtained nasal swab samples for rhinovirus screening from 169 children ⩾1 month of age who were hospitalized for any reason on the pediatric infectious diseases ward of Turku University Hospital (Turku, Finland). The mean interval between screening and discharge from the hospital was 0.6 days (range, 0–2 days) for rhinovirus-positive children and 1.1 days (range, 0–3 days) for rhinovirus-negative children. At the time of discharge of index children positive or negative for rhinoviruses, family members living in the same household were recruited for the study. Families with only 1 child were excluded from the study. The parents documented respiratory symptoms (i.e., rhinorrhea, nasal congestion, cough, and sore throat) and fever in the family members in a diary throughout a 3-week follow-up period. The investigation was conducted during September–November, when rhinovirus prevalence in the community was high. The study protocol was approved by the Ethics Committee of Turku University Hospital. Written informed consent was received from all adult participants; parents provided consent on behalf of nonadult participants.

Virologic analyses. A research nurse taught a parent the technique of obtaining a nasal swab sample from a depth of 2–3 cm in the nose by use of a sterile cotton swab, and the parent obtained samples from all family members twice weekly throughout the 3-week follow-up period. The swabs were sent in dry sterile tubes to the laboratory by mail and were stored at −70°C until analyzed. Preliminary studies showed that sample storage at ambient indoor temperature for up to 4 days or sample submission by mail (transit time, 1–2 days) had only slight, if any, effect on the recovery rate or copy numbers of rhinoviruses detected by the RT-PCR assay (data not shown).

The nasal swabs were vortexed in 1 mL of PBS, and RNA was extracted from 150-μL volume, using the E.Z.N.A. Viral RNA Isolation Kit (Omega Bio-Tek) according to the manufacturer's protocol. The specimens were analyzed using a multiplex real-time RT-PCR for rhinoviruses, enteroviruses, and respiratory syncytial virus. Rhinovirus RT-PCR amplification was performed with conserved (4− and 3+) picornavirus primers from the 5' noncoding region of the genome [30]. RNA isolated from purified rhinovirus 1B was used as a positive control. PCR reactions were performed in a RotorGene 3000 instrument (Corbett Life Sciences) in 25-μL reactions containing 5 μL of the RT reaction product, QuantiTect SYBR Green PCR mix (Qiagen), and 600 nmol/L of picornavirus and respiratory syncytial virus primers according to the following procedure: 95°C for 15 min, followed by 45 cycles at 95°C for 15 min; 65–55°C for 30 s (touchdown 1°C/cycle for the first 10 cycles), and 72° for 40 s (melt 72°C–95°C, 0.5°C/s). Positive amplicons were identified as rhinoviruses, enteroviruses, or respiratory syncytial virus according to melting temperatures. Rhinovirus RT-PCR–positive amplification products were further differentiated by melting temperatures. Groups of RT-PCR products with nonoverlapping mean melting temperatures (±2 standard deviations [SDs]) (⩾0.9°C difference between the amplicons) were considered to represent separate virus types, provided that sequence analysis supported this grouping.

Quantitative RT-PCR was performed with the picornavirus primers as described above, using dilutions of human rhinovirus 16 RNA from purified virions with a spectrophotometrically determined copy number as a standard. The dynamic range of the assay was 10^4–10^{10} viral genome copies/sample, and the sensitivity was 1000 copies/sample. The SD for 5.6-$\log_{10}$ copies of the HRV1B positive control RNA in 19 runs was ±0.26 $\log_{10}$ copies. For sequence analysis, rhinovirus-positive cDNA was amplified as described above with another combination of 4− and 2+ (5'-CAAGCACTTCTGTTTCCCC) 5' noncoding region primers generating 397-bp long amplicons, which were purified using the QIAquick PCR purification kit (Qiagen) and sequenced in the DNA Sequencing Service Laboratory of the Turku Center for

Table 1. Demographic data and incidence of rhinovirus infections in family members during a 3-week follow-up period.

Infection status of index child, study subject	No. of subjects	Age, median (IQR), years	No. of males; no. of females	No. of infections (per-person rate)		
				Symptomatic	Asymptomatic	Overall
Rhinovirus positive						
Index children	8	1.3 (0.6–1.5)	7; 1	8 (1.00)	1 (0.13)	9 (1.13)
Siblings	17	8.6 (6.2–10.3	10; 7	12 (0.71)	5 (0.29)	17 (1.00)
Parents	14	32.7 (31.4–37.3)	6; 8	2 (0.14)	5 (0.36)	7 (0.50)
Rhinovirus negative						
Index children	16	4.3 (1.0–8.6)	12; 4	5 (0.31)	3 (0.19)	8 (0.50)
Siblings	24	7.4 (3.4–11.4)	10; 14	8 (0.33)	5 (0.21)	13 (0.54)
Parents	30	36.1 (30.8–39.9)	14; 16	5 (0.17)	2 (0.07)	7 (0.23)

NOTE. IQR, interquartile range.

Biotechnology. The sequences are available from the authors on request.

The partial 5' nucleotide sequences of the viruses were aligned using ClustalW software, version 1.82 [31], and adjusted manually to equal lengths (332 bp), using the SeaView editor. Relationships of the aligned sequences were inferred using programs of the Phylip Package, version 3.65 [32], and distance matrices and similarity tables were calculated using the DNADIST program with the F84 model and a transition/transversion ratio of 2.0. Dendrograms were constructed using the neighbor-joining option in the Phylip Package, using 101 Jumbles. Support for tree topology was estimated by bootstrap analysis, using SEQBOOT with 100 replicates, and the consensus tree was calculated by means of CONSENSE. Phylogenetic trees were visualized with Mega 3.1 [33]. Comparison with the sequences of 17 rhinovirus reference strains led to the conclusion that viruses with >98% similarity represent the same virus type.

Statistical analysis. We used multinomial logistic regression analysis to model whether detection of rhinovirus in the index child, age of individuals (<7 years, 7–16 years, and ⩾17 years), or interaction of these 2 parameters (at the level of $P < .1$) was associated with the incidence of rhinovirus infection among family members. The inclusion of age as a risk factor was based on earlier studies suggesting that rhinovirus is most efficiently transmitted to young children [1, 2, 18]. In addition, the association of rhinovirus detection in the index child with rhinovirus infections in family members was analyzed separately for the 3 age groups. Cumulative odds ratios (CORs) were calculated for the risk factors. Odds ratios were also analyzed for the association between rhinovirus detection in asymptomatic subjects and symptomatic or asymptomatic rhinovirus infection in the same individual or in any other family member. Viral copy numbers were compared between samples from symptomatic and asymptomatic individuals by use of the Mann-Whitney *U* test. Logistic regression analysis was used to compare the percentage of rhinovirus-positive samples with the percentage of rhinovirus-negative samples, as well as the frequencies of rhinovirus-positive samples with $\leqslant 10^4$, 10^4–10^5, 10^5–10^6, or $>10^6$ genomes detected, during the early symptomatic period (days 0–4), the late symptomatic period (days 5–21), and the postinfection period (days 1–7 after symptom resolution). $P < .05$ was considered statistically significant. Data analyses were performed using SAS software (SAS Institute).

RESULTS

Characteristics of the study subjects. Eight families (consisting of 39 individuals) with a rhinovirus-positive index child and 16 families (consisting of 70 individuals) with a rhinovirus-negative index child completed the follow-up documentation of respiratory symptoms and sent nasal swab samples to the laboratory. At the time of hospitalization, 6 rhinovirus-positive index children (75%) and 9 rhinovirus-negative index children (56%) had symptoms of respiratory infection (i.e., wheezing illness, pneumonia, croup, or common cold). Table 1 shows demographic and clinical characteristics of the study subjects. Overall, 467 nasal swab samples were studied.

Incidence of rhinovirus infection among family members. During the 3-week follow-up period, the incidence of any rhinovirus infection (symptomatic or asymptomatic) was 1.00 cases per sibling and 0.50 cases per parent in households with a rhinovirus-positive index child (table 1). Rhinovirus infection was less frequent in families with a rhinovirus-negative index child, with an incidence of 0.54 cases per sibling and 0.23 cases per parent.

The incidence of rhinovirus infection (both symptomatic and asymptomatic) among study subjects was associated with rhinovirus detection in the index child (COR, 7.4; 95% confidence interval [CI], 1.9–28.6; $P = .004$) and age of 0–16 years ($P = .002$), but no interaction was seen between these 2 parameters ($P = .26$) (table 2). However, among rhinovirus infections with clinical symptoms, interaction was seen between age and virus detection in the index child ($P = .09$). The number of these infections was associated with low age ($P = .0005$), but

Table 2. Rhinovirus infection rates, by age of study subjects, during a 3-week follow-up period.

Infection type, age	No. of infections/no. of subjects (no. of infections/subject), by infection status of index child			COR (95% CI)[a]
	Positive	Negative	Overall	
Symptomatic				
<7 years	12/13 (0.92)	9/20 (0.45)	21/33 (0.64)	14.3 (3.9–52.3)
7–16 years	8/12 (0.67)	4/20 (0.20)	12/32 (0.38)	3.6 (0.9–13.6)
>16 years (adult)	2/14 (0.14)	5/30 (0.17)	7/44 (0.16)	1.0
Asymptomatic				
<7 years	2/13 (0.15)	0/20 (0)	2/33 (0.06)	ND
7–16 years	4/12 (0.33)	8/20 (0.40)	12/32 (0.38)	2.9 (0.9–10.1)
>16 years (adult)	5/14 (0.36)	2/30 (0.07)	7/44 (0.16)	1.0
Overall				
<7 years	14/13 (1.08)	9/20 (0.45)	23/33 (0.70)	5.3 (1.8–16.0)
7–16 years	12/12 (1.00)	12/20 (0.60)	24/32 (0.75)	6.4 (2.1–19.9)
>16 years (adult)	7/14 (0.50)	7/30 (0.23)	14/44 (0.32)	1.0

NOTE. CI, confidence interval; COR, cumulative odds ratio; ND, not determined.

[a] By multinomial logistic regression.

there was no statistically significant association with virus detection in the index child (COR, 3.1; 95% CI, 0.7–13.9; $P = .13$). Because asymptomatic rhinovirus infections were rare among siblings <7 years of age, this age group was not included in the statistical analysis. Asymptomatic rhinovirus infection in the other age groups fit the interaction model ($P = .06$). In this model, neither the association with age group ($P = .09$) nor with rhinovirus infection in the index child were significant (COR, 2.5; 95% CI, 0.6–10.2; $P = .18$). In a separate analysis of adults, rhinovirus detection in the index child was a risk factor for asymptomatic infection ($P = .05$) but not for symptomatic infection ($P = .85$). Taken together, the data show that rhinoviruses were transmitted efficiently within the families and that most infections (21 of 23) in young children were symptomatic but that approximately half of infections (19 of 38) in older children and adults were asymptomatic.

Source of asymptomatic rhinovirus infections. To identify the source of rhinovirus detected in asymptomatic individuals, we analyzed connections between these findings and infections in the same individual or in the family. One or more family members of 32% of asymptomatic subjects positive for rhinovirus had a simultaneous symptomatic rhinovirus infection, compared with 17% of subjects negative for rhinovirus (OR, 2.5; 95% CI, 1.1–5.8; $P = .03$). Symptomatic infections in the family were also frequent before (38%; $P = .09$) but not so common after (23%; $P = .38$) detection of rhinovirus in an asymptomatic subject. Detection of rhinovirus in asymptomatic individuals was not significantly associated with asymptomatic infection in other family members, nor was it associated with symptomatic infection in the same subjects during the follow-up period.

Rhinovirus genome copy numbers. The median copy number of rhinovirus was 5.3 $\log_{10}$ copies/sample (interquartile range, 4.3–6.1 $\log_{10}$ copies/sample) for 49 nasal swab specimens obtained when respiratory symptoms were present and 4.9 $\log_{10}$ copies/sample (IQR, 4.1–5.9 $\log_{10}$ copies/sample) in 44 specimens obtained when no symptoms were present ($n = 44$ persons) ($P = .41$, by the Mann-Whitney U test). Positive RT-PCR results were observed more frequently for samples obtained during the early phase of infection (19 [86%] of 22 samples), compared with samples obtained during the late phase of infection (20 [33%] of 60) or during the postinfection period (9 [27%] of 33) ($P = .0009$) (figure 1). No significant difference was seen in the relative viral copy numbers in relation to the duration of respiratory symptoms ($P = .43$).

Rhinovirus typing by melting temperature and sequence analysis. RT-PCR products from families with rhinovirus infections were first grouped by the melting temperatures, and 5' noncoding regions of high copy number RT-PCR products were sequenced, aligned, and subjected to phylogenetic analysis (figure 2). Viruses with >98% similarity were considered to be representatives of the same virus genotype. Twelve rhinovirus genotypes were identified. Figure 3 shows the association of virus detection, copy numbers, virus type according to melting temperature and sequence comparison, and clinical symptoms in 4 families. The same rhinovirus genotypes were detected in several family members simultaneously or sequentially (e.g., family 2), indicating transmission within the family. Asymptomatic infection, probably acquired from the young children, was seen in adults and older children. Several different virus strains were frequently identified within the families (e.g., families 10, 51, and

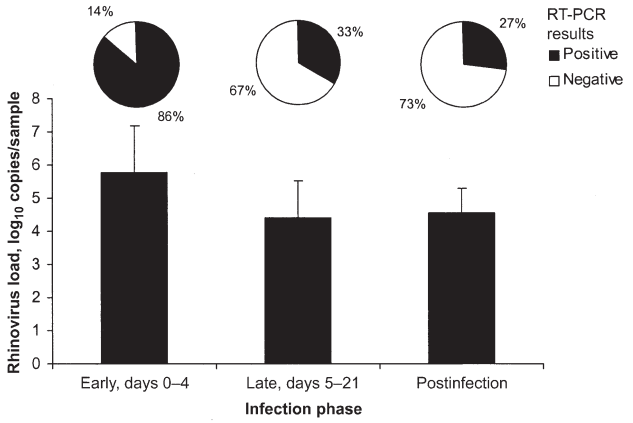

Figure 1. Detection and median quantity (±SD) of rhinovirus, according to duration of respiratory symptoms. Postinfection samples were from the 7-day period after the end of respiratory symptoms. The odds ratio for detection of rhinovirus during the early infection phase was significantly greater than that during the late and postinfection phase ($P = .0009$, by logistic regression analysis). Data are from the whole study population, and all rhinovirus findings regardless of the virus type have been included. Thus, postinfection results can include newly acquired asymptomatic infections in addition to carriage of virus after symptoms. RT-PCR, reverse transcription–polymerase chain reaction.

61), although the follow-up period was not longer than 3 weeks. Sequential infections with different rhinovirus strains also occurred in individual subjects.

DISCUSSION

We found a high incidence of rhinovirus infection in families with children during the autumn season. Rhinoviruses were detected in virtually all children and in half of adults in families with a rhinovirus-positive index child. This finding is put in context by the detection of 0.5 rhinovirus infections per sibling and 0.2 infections per parent during the follow-up period in families with a rhinovirus-negative index child. The melting temperature and sequence analysis showed the simultaneous circulation of several rhinovirus types within families. The overlapping symptomatic periods caused by different rhinovirus types and the occurrence of infections before the follow-up period make it difficult to calculate exact transmission rates for rhinoviruses in this study. However, comparisons between the incidence of asymptomatic and symptomatic infections in families with a rhinovirus-positive or rhinovirus-negative index child show that the transmission efficiency is high but that the contagion of virus does not always lead to clinical symptoms.

Rhinovirus infections are most frequent in children [1, 2, 18]. In a study of families conducted during 1965–1966, infection was usually transmitted from school-aged children to other family members [19]. In more-recent studies involving RT-PCR, rhinoviruses have also been frequently detected in asymptomatic individuals, with the highest incidence among children [28, 29]. Our results partly agree with the earlier findings and extend them further. We found that most infections in young children were symptomatic and that approximately half of infections among older children and adults were asymptomatic. Of interest, detection of rhinovirus in an index child was a risk factor for an asymptomatic, but not symptomatic, infection in the parents. These data show that rhinoviruses are efficiently transmitted in families but that adults are often protected from symptomatic infection, probably by acquired immunity, because most have serum antibodies against many rhinovirus types [17]. Other host

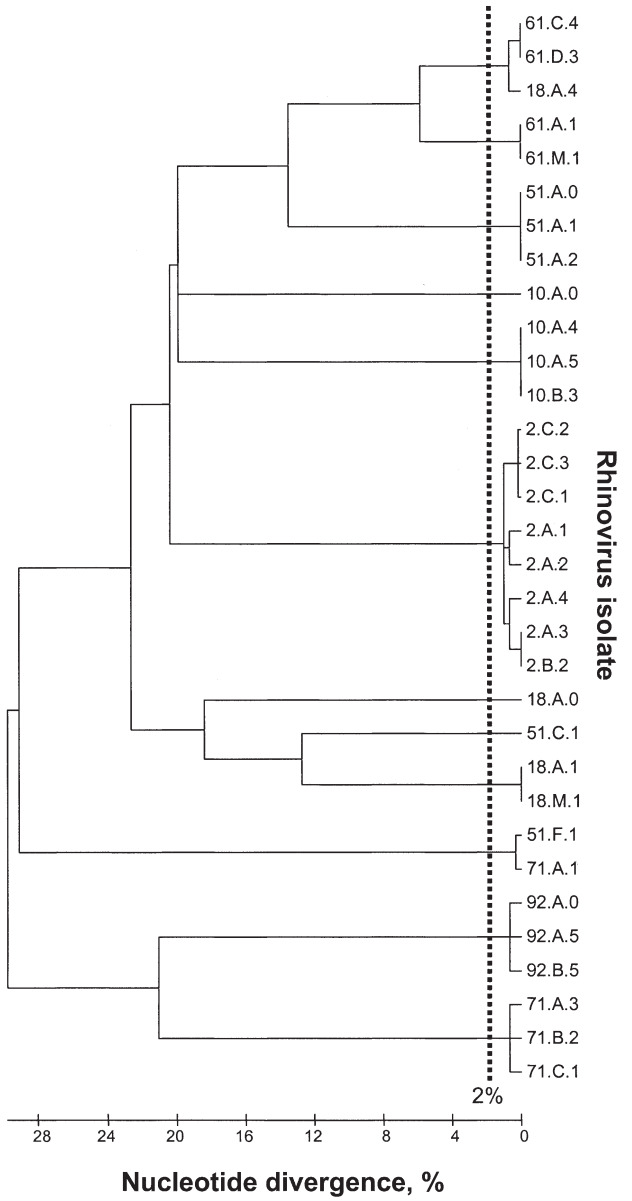

Figure 2. A linearized tree demonstrating nucleotide sequence divergence between 5' ends (332 bp) of rhinoviruses isolated from study subjects. Virus samples are coded by family number, family member (A–D, children; M, mother; and F, father), and sequential sample number. *Dashed line,* divergence of <2% for the rhinovirus strains that are considered to be of the same type.

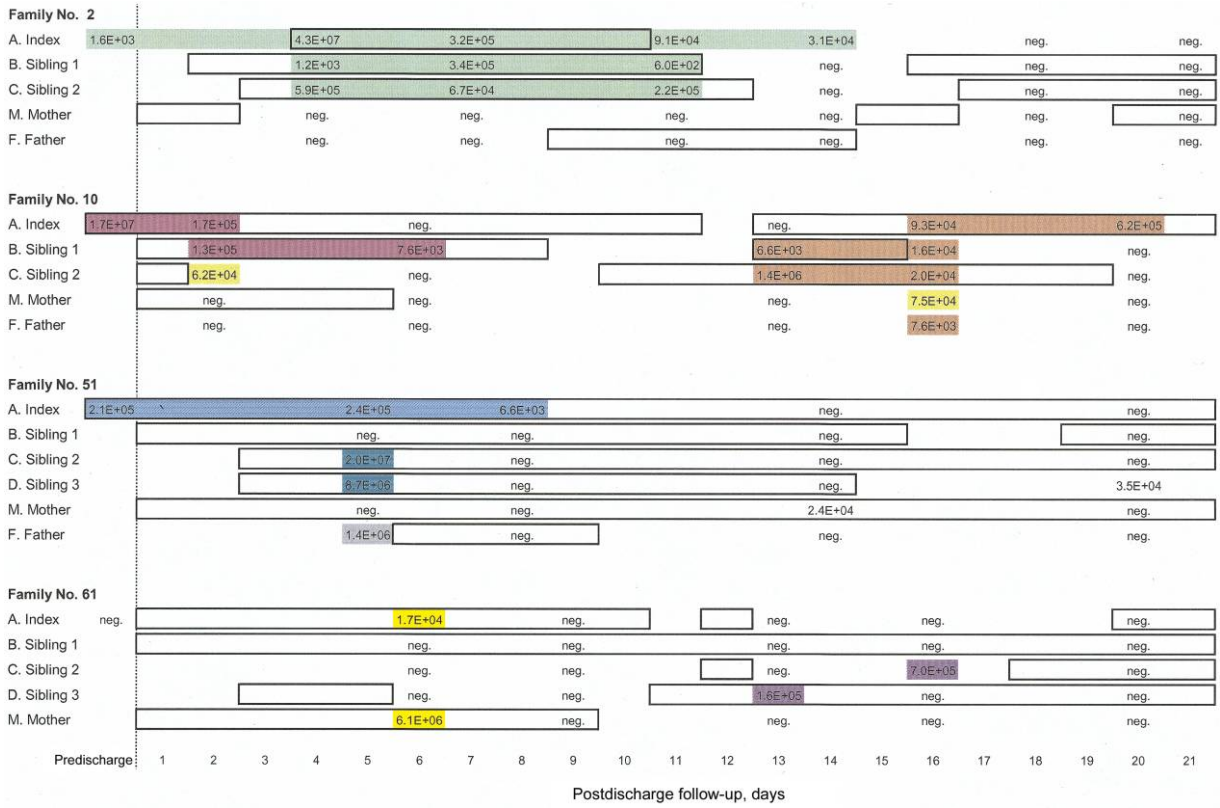

Figure 3. Durations of respiratory symptoms *(boxes)* and rhinovirus copy numbers (in log values per sample) during a 3-week follow-up period in 4 families. Colors represent virus strains, differentiated by the melting temperature and sequence analysis. Several strains circulated within the families simultaneously or sequentially. Rhinovirus loads inside boxes indicate that virus was recovered during symptomatic infection; rhinovirus loads outside of boxes indicate that virus was recovered during asymptomatic infection.

factors may also play a role in the development of symptomatic infection after exposure to virus. The major receptor for rhinoviruses—intracellular adhesion molecule (ICAM)–1—is upregulated in the respiratory tract of adults with asthma, and people with asthma have more severe rhinovirus infection than do healthy people [34], but there are no data comparing ICAM-1 expression between children and adults. Differences between rhinovirus types, expression of currently unknown host factors regulating virus replication in the cells, and different actions of components of the immune system may further explain these findings. The present study was conducted during high epidemic activity of rhinoviruses in families with at least 2 children. Intense exposure to rhinoviruses may explain why most infections in young children were symptomatic. In other epidemic settings, we would expect to see asymptomatic infections also in young children. Another explanation for the discrepancy between findings from the present study and those from earlier studies is that mild symptoms may easily go unnoticed and that, as a result, some studies may have overestimated the frequency of asymptomatic infections.

Nasal samples can be positive for rhinovirus for up to 5 weeks after a symptomatic infection [27]. This finding has been attributed to the carriage of virus after infection, but the acquirement of a new, asymptomatic infection has not been ruled out. In our study, carriage of virus at least for a short term after the symptomatic period did occur. However, rhinovirus findings in asymptomatic individuals were infrequently associated with a preceding symptomatic infection, whereas a significant association was detected with simultaneous rhinovirus infections in other family members. Our data shows that many rhinovirus infections in adults and older children are truly asymptomatic and acquired from young children in the family. We did not find evidence of asymptomatic individuals transmitting virus to their contacts, suggesting that respiratory symptoms are likely necessary for the efficient spread of rhinovirus. However, other studies would be needed to corroborate these findings, because of the risk of contamination when parents collect samples from all household members.

Rhinoviruses were detected most efficiently during the early phase of infection. This is in agreement with results of experimental infection studies that detected the highest amounts of rhinovirus in nasal washings during the first 3 days of symptoms [35]. After this period, the presence of rhinoviruses was no longer correlated with the occurrence of respiratory symptoms. We expected that viral copy numbers in symptomatic persons would be higher than those in asymptomatic persons, but no

such difference was detected, suggesting that variation between virus types and/or differences in the effects of host factors between infected individuals play a significant role in pathogenesis. Nasal swab sampling by parents is subject to considerable variation in the amount of sample collected, which also causes variation in the viral copy numbers per sample. Detection of high viral loads also in asymptomatic individuals agrees with a recent study recommending caution in association of rhinovirus PCR positivity with clinical illness [36].

To define exact transmission chains, rhinovirus types have to be differentiated from each other. Since the current RT-PCR methods applied to clinical nasal specimens are not sufficiently sensitive for amplification of rhinovirus VP1 gene sequences, which are known to correlate well with the serotype concept, we amplified a 0.4-kb fragment from the 5'-end of viral genomes with conserved primers. The PCR product was used for identification of strains, using melting temperature analysis and also sequencing whenever possible. When tested with rhinovirus reference strains, the combination of these methods turned out to be applicable to the identification of virus types. In agreement with findings from earlier studies [1, 16, 17], we demonstrated simultaneous circulation of several rhinovirus types in the community. Of note, up to 3 rhinovirus types were simultaneously detected in the members of 1 household. Consequently, the high infection rates in families with a rhinovirus-positive index child represent both the transmission of a virus infecting the index child and concomitant circulation of other rhinoviruses.

In summary, this study demonstrates the high extent and high diversity of rhinovirus circulation in families during the autumn season. Self-collected nasal swab samples are suitable for semiquantitative detection and molecular epidemiology studies of rhinoviruses. Rhinoviruses are transmitted efficiently between siblings and cause symptoms in most young children. Asymptomatic infections are common in older children and adults, usually representing transmission from young children. The mechanisms that determine the appearance of clinical symptoms are probably of both viral and host origin, and their role in the pathogenesis of rhinovirus disease will require further studies, using multiple approaches. Efforts to prevent rhinovirus transmission should be focused on young children with respiratory symptoms, because of their central role in the spread of virus.

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