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Presence of Specific Viruses in the Middle Ear Fluids and Respiratory Secretions of Young Children With Acute Otitis Media

Johanna Nokso-Koivisto,[1,3]* Riitta Räty,[1] Soile Blomqvist,[1] Marjaana Kleemola,[1] Ritva Syrjänen,[2] Anne Pitkäranta,[3] Terhi Kilpi,[2] and Tapani Hovi[1]

[1]*Department of Microbiology, National Public Health Institute, Helsinki, Finland*
[2]*Department of Vaccines, National Public Health Institute, Helsinki, Finland*
[3]*Department of Otorhinolaryngology, Helsinki University Central Hospital, Helsinki, Finland*

The purpose of the study was to investigate the presence of different viruses in middle ear fluids and nasopharyngeal aspirates in young children with acute otitis media. Two cohorts of children (N = 329 and 611) were followed from 2 to 24 months of age in Finland in two prospective studies (Finnish Otitis Media Cohort Study and Finnish Otitis Media Vaccine Trial). During the study period, nasopharyngeal and middle ear fluid specimens for each acute otitis media event were examined for eight (Cohort Study) or ten (Vaccine Trial) common respiratory viruses; adenoviruses, influenza viruses A and B, parainfluenza viruses 1, 2, and 3, respiratory syncytial virus (RSV), enteroviruses, parechoviruses, and rhinoviruses. Picornaviruses (rhinoviruses, enteroviruses, and parechoviruses) were determined by reverse transcription PCR while antigen detection was used for the other viruses. A virus was present in either nasopharyngeal or middle ear specimen in 54% of events in the first cohort and in 67% of events in the second. Rhinoviruses formed the most common virus group detected (41–32%), followed by enteroviruses (25%, sought in the second cohort only) and respiratory syncytial virus (RSV) (10%). All the other viruses represented jointly 8–10% of the events. In conclusion, using the methods described in this study, a specific virus infection was diagnosed in two thirds of all acute otitis media events in young children. Picornavirus RNA was detected in association with more than a half of all acute otitis media events. The use of PCR-based methods for the other respiratory viruses might have increased further the overall virus detection rate in acute otitis media. ***J. Med. Virol. 72:241–248, 2004.*** © 2004 Wiley-Liss, Inc.

KEY WORDS: respiratory virus; rhinovirus; enterovirus; RSV; PCR; TR-FIA

INTRODUCTION

Acute otitis media is generally regarded as a bacterial infection. However, many studies over the past decades have shown the close association between acute otitis media and respiratory viral infections. This association has been demonstrated by temporal coincidence of viral epidemics and peak incidences of acute otitis media in epidemiological studies [Henderson et al., 1982] as well as documenting viral infections in acute otitis media patients [Chonmaitree et al., 1986; Heikkinen et al., 1999] and it was already evident in studies exploiting conventional techniques, i.e., isolation of viruses from nasal aspirates and middle ear fluid samples or by using serology [Chonmaitree et al., 1986; Harsten et al., 1991]. The development of new simpler and often more sensitive laboratory methods for virus detection, such as antigen assays and polymerase chain reaction (PCR) assays, has contributed to the accumulation of further evidence of this association [Pitkäranta et al., 1998; Heikkinen et al., 1999]. The most convincing evidence is based on virus specific intervention studies: a proportion of acute otitis media occurring during influenza outbreaks can be prevented by immunizing children with influenza virus vaccine [Heikkinen et al., 1991].

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*Correspondence to: Johanna Nosko-Koivisto, Department of Microbiology, National Public Health Institute, Mannerheimintie 166, 00300 Helsinki, Finland.
E-mail: johanna.nosko.koivisto@ktl.fi

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With modern diagnostic methods human rhinovirus group and RSV have been found to be the most common respiratory viruses associated with acute otitis media in young children [Arola et al., 1990; Pitkäranta et al., 1998; Vesa et al., 2001]. Human rhino-, entero-, and parechoviruses are RNA viruses and belong to the Picornaviridae family. Rhinoviruses are assumed to cause mostly mild upper respiratory infections, although more severe diseases such as pneumonia have also been documented [El-Sahly et al., 2000]. For human enteroviruses, the clinical expressions of infection range from severe paralysis to minor febrile illnesses [Chonmaitree and Mann, 1995]. Enteroviruses have been found by virus culture in up to 6% of middle ear fluid samples obtained from patients with acute otitis media [Chonmaitree et al., 1986; Heikkinen et al., 1999]. Cell lines used for viral culture vary in their sensitivity to different enteroviruses and some enterovirus serotypes cannot be isolated in standard cell cultures used in diagnostic laboratories [Pallansch and Roos, 2001]. The introduction of the reverse transcription (RT)-PCR method has made it possible to study systematically the role of enteroviruses in acute otitis media in children. Parechoviruses, previously classified among enteroviruses as echovirus serotypes 22 and 23, are also known to be respiratory pathogens [Joki-Korpela and Hyypiä, 2001].

In two Finnish studies, the Finnish Otitis Media Cohort Study and Vaccine Trial, large groups of children were followed closely for the occurrence of acute respiratory infections and acute otitis media from 2 to 24 months of age over a total period of 5 years. Virological results from the Cohort Study have been reported in part earlier [Vesa et al., 2001; Blomqvist et al., 2002; Nokso-Koivisto et al., 2002b]. In this study, we present virological findings from both study groups based on specimens collected during acute otitis media in 940 children, as well as an analysis of seasonal variation of specific virus occurrence covering a time period from April 1994 through August 1998.

SUBJECTS

Study Groups and Study Design

The present study included children from two separate cohorts. The first study group comprised 329 healthy children who were enrolled in the Finnish Otitis Media Cohort Study. The purpose of the Cohort Study was to assess the epidemiology and risk factors of acute otitis media in children during the follow-up period from 2 to 24 months of age from April 1994 through July 1997. The study design has been presented in detail elsewhere [Syrjänen et al., 2001; Vesa et al., 2001].

The other study group was derived from the Finnish Otitis Media Vaccine Trial, which was a prospective randomized and double blind cohort study designed for evaluation of the efficacy and safety of two sevenvalent pneumococcal conjugate vaccines. The study was conducted from December 1995 through March 1999 and altogether 2,497 children were enrolled. The study design and the efficacy results for the conjugate vaccines have been published elsewhere [Eskola et al., 2001]. All specimens collected from a sample of children were subjected to virological testing. The sample comprised 611 subjects who were randomly selected from among the 1,610 children recruited during the first year of the trial and whose follow-up time was from December 1995 through September 1998. They did not differ significantly from the rest of the children with regard to date of birth, sex, or any other aspect.

In both studies, the children enrolled were followed in study clinics established specifically for the purpose in the Tampere region in Finland, with a specially trained study doctor and nurse in each clinic. All study children visited the clinic for enrollment at 2 months of age, and thereafter seven or nine times, depending on the study, until the age of 24 months. Physical examination was carried out and interview data were collected during these scheduled visits. In addition, the families were encouraged to take the child to the study clinic whenever he or she had symptoms of acute respiratory infection, and especially if acute otitis media was suspected. If an acute otitis media was diagnosed by the study doctor, myringotomy was performed and middle ear fluid specimens were drawn from the inflamed ear(s) with an electric suction apparatus into a sterile collector. In addition, a nasopharyngeal aspirate was obtained using a sterile pediatric mucus extractor.

An informed written consent was obtained from the parents of the study children. The study protocols and consent forms were approved by the Ethical Committee of the National Public Health Institute and the Ethical Committees of Tampere Health Center and Tampere University Hospital.

Definitions

A child was considered to have acute otitis media if she or he had a visually abnormal tympanic membrane (in regard to colour, position, and/or mobility) suggesting the presence of middle ear fluid, together with at least one of the following signs or symptoms of acute infection: fever, ear ache, irritability, diarrhea, vomiting, acute otorrhea not caused by otitis externa, or other simultaneous respiratory tract symptoms [Karma et al., 1987]. The current testing includes all acute otitis media events for which both the nasopharyngeal aspirate sample and at least one middle ear fluid sample were available for virological examination (evaluable acute otitis media events). These represent 87% of all acute otitis media events both in the Cohort Study and in the Vaccine Trial.

A virus positive acute otitis media event was an evaluable acute otitis media event with any virus or viruses detected in the nasopharyngeal aspirate or middle ear fluid specimen, or both.

SPECIMENS AND LABORATORY METHODS

For both the Cohort Study and the Vaccine Trial, the nasopharyngeal aspirate and middle ear fluid specimens were frozen immediately after collection and

stored at −70°C before testing. For the current study, all specimens collected at evaluable acute otitis media events occurring during the Cohort Study, and the corresponding specimens derived from a sample of 611 children of the Vaccine Trial were subjected to virological tests. In the Finnish Otitis Media Cohort Study, rhinoviruses were detected by a combined isolation-RT-PCR method reported previously [Blomqvist et al., 1999].

Antigen Detection

The one-incubation time-resolved fluoroimmunoassay (TR-FIA) [Halonen et al., 1983; Hierholzer et al., 1987, 1989; Nikkari et al., 1989] was used for the detection of influenza virus A and B, parainfluenza virus types 1, 2, and 3, RSV, and adenovirus specific antigens from nasopharyngeal aspirate and middle ear fluid samples. The cut-off value for the positivity was the mean of the negative controls plus six times the standard deviation of the mean.

Multiplex RT-PCR-Hybridization Method for Picornavirus Detection

In the Finnish Otitis Media Vaccine Trial, the nasopharyngeal aspirate and middle ear fluid specimens were analyzed for rhinovirus, enterovirus, and parechovirus RNA by a modification of the RT-PCR-hybridization method described previously [Blomqvist et al., 1999]. RNA was extracted from 100 μl of nasopharyngeal aspirate or middle ear fluid using a commercial kit (RNeasy®, Qiagen, GmbH, Heidelberg, Germany). Five microlitres of RNA was reverse-transcribed in 96-well plates (Stratagene, GmbH, Heidelberg, Germany) in a final volume of 40 μl, simultaneously with one reverse primer for rhino- and enteroviruses and one for parechoviruses. Five microlitres of cDNA was subjected to PCR in 96-well plates with the two reverse primers and two biotinylated forward primers, one for rhino- and enteroviruses and one for parechoviruses.

Primers and probes for rhino- and enteroviruses have been published previously [Blomqvist et al., 1999; Nokso-Koivisto et al., 2002a]. For parechoviruses, the primers were 5′-CCCAGATCAGATCCACAG-3′ (reverse), 5′-TGCCCCTGGGGCCAAAAG-3′ (forward), and the probe was 5′-AACCAATCCTAA(A/T)GGGTC-TG-3′. Three prototype viruses (human rhinovirus 2, coxsackievirus A16, and parechovirus 2) were used as positive controls. At each step, several negative controls were included.

The PCR amplicons were identified by a microplate hybridization method [Blomqvist et al., 1999] with three lanthanide-labeled probes (Wallac Oy, Turku, Finland), whose specifities were tested with all known prototype strains of rhinoviruses, enteroviruses, and parechoviruses. Europium-labeled rhinovirus probe detected 96 out of 102 rhinovirus prototypes, but none of the 64 enteroviruses or two parechoviruses. Samarium-labeled enterovirus probe hybridized with all 64 enteroviruses, with 38 out of 102 rhinoviruses and neither of the parechoviruses. There were five rhinovirus prototypes (human rhinoviruses 45, 51, 65, 71, and 87), which were negative for the rhinovirus probe, but positive for the enterovirus probe. One rhinovirus prototype (type 12) was negative for both probes. Terbium-labeled parechovirus probe recognized the two parechovirus prototypes, but none of the rhino- or enterovirus prototypes. All PCR amplicons were tested with three different probes at the same time in three parallel wells in a 96-well plate.

The cut-off value for positive specimens was ten times the mean of all negative controls for the Europium-labeled probe [Halonen et al., 1995], and for the Samarium- and Terbium-labeled probes the mean of all the negative controls plus five times the standard deviation of the mean modified from Heinonen et al. [1997]. A positive result in at least two of the three parallel wells was required to score a specimen positive. The specimen was regarded rhinovirus positive if the rhinovirus probe yielded a positive result and enterovirus positive, if the enterovirus probe alone was positive. However, because of the aberrant reaction of a few rhinovirus prototypes, our "enterovirus" group may include some rhinoviruses. Nevertheless, for simplicity these viruses are later called enteroviruses. Parechovirus was detected if the parechovirus probe gave a positive result.

RESULTS

In these two studies, altogether 940 children were followed up and examined for evidence of viral infection each time they were diagnosed with acute otitis media at the study clinic. The rates of any acute otitis media events and evaluable acute otitis media events were very similar in both Finnish Otitis Media Cohort Study and Vaccine Trial. The general characteristics of the two cohorts are presented in Table I.

Viral Infections Associated With Acute Otitis Media

In the Finnish Otitis Media Cohort Study, a total of 203 children out of 329 (62%) experienced 759 evaluable acute otitis media events (range 1–16). Altogether 406 (54%) of these events were positive for at least one virus and in 34 (4.5%) events two viruses were found. The virus or viruses were identified in the nasopharyngeal aspirate sample in 322 (42%) events, and in at least one middle ear fluid sample in 252 (33%) events. Rhinovirus was detected most frequently, in 309 (41%) of the evaluable acute otitis media events. RSV was detected in 70 (9%) acute otitis media events, influenza virus A in 20 (3%), adenovirus in 20 (3%), and parainfluenza virus 3 in 17 (2%) events. Parainfluenza virus 1 was found only once, parainfluenza virus 2 three times, and not a single sample was positive for influenza virus B. The rates of different virus events per person year are presented in Table II. Enteroviruses or parechoviruses were searched for in the Vaccine Trial only.

In the Finnish Otitis Media Vaccine Trial, 459 children out of the 611 study children (75%) experienced

TABLE I. General Characteristics of the two Cohorts of Children With Acute Otitis Media (AOM) Followed Prospectively in the Finnish Otitis Media (FinOM) Cohort Study and in the FinOM Vaccine Trial

	FinOM Cohort Study N (%[a])	FinOM Vaccine Trial N (%[a])
Study group for virological studies	329 children (53% of the source population)	A random sample of 611 children (out of 1,610 children representing 55% of the source population)
Female/male	171 (52%)/158 (48%)	295 (48%)/316 (52%)
Dropouts	48 (15%)	10 (2%)
Study period	April 1994–July 1997	December 1995–September 1998
Rate of any AOM events/person year	1.54	1.47
Rate of evaluable AOM events[b]/person year	1.34	1.28
Children without any acute otitis media[c]	88 (31%)	136 (23%)

[a]% of the corresponding study group.
[b]With both nasopharyngeal aspirate and at least one middle ear fluid sample available for virus detection.
[c]Among the children followed up to 2 years of age (dropouts excluded).

in total 1,416 evaluable acute otitis media events (range 1–14) during the follow-up. Nine hundred and fifty five events (67%) were positive for at least one virus, and 142 (10%) were positive for two or three viruses. The nasopharyngeal aspirate sample was virus positive in 892 (63%) events and one or both of the middle ear fluid samples in 579 (41%) events. Picornaviruses were found to be associated with more than half of the acute otitis media events (54%), rhinoviruses were found in 453 (32%) events, and a positive result was obtained with the enterovirus probe alone in 360 (25%) events while parechoviruses were seen only rarely (1%). RSV was found in 135 (10%) acute otitis media events and parainfluenza virus 3 in 58 (4%) events. The incidence of other viruses was less than 3% each. The rates of different virus events per person year are shown in Table II.

Concurrent Virus Detection in Nasopharyngeal Aspirate and Middle Ear Fluid Samples

In the Finnish Otitis Media Cohort Study, 848 nasopharyngeal aspirate and 1,088 middle ear fluid specimens collected at the time of acute otitis media were available for virological tests. Out of the 406 virus positive acute otitis media events, 168 events (41%) were nasopharyngeal aspirate and middle ear fluid positive for any virus concurrently, and in 163 events (40%) the same specific virus was detected concurrently in both specimen types (Table III). RSV was detected concurrently in both types of samples in 59% of the RSV positive events, while the concurrence for rhinoviruses was 36% and for influenza virus A 40% (Table III). It is noteworthy that at 86 events rhinovirus was detected in middle ear fluid yet not simultaneously in nasopharyngeal aspirate.

In the Finnish Otitis Media Vaccine Trial, 1,491 nasopharyngeal aspirate and 2,122 middle ear fluid specimens collected at the time of acute otitis media were analyzed for viruses. Nasopharyngeal aspirate and middle ear fluid were positive for any virus concurrently in 516 events (54% out of 955 virus positive events), and in 466 events (49%) the same virus was detected concurrently in the nasopharyngeal aspirate sample and in at least one of the middle ear fluid specimens

TABLE II. Rates of Evaluable Acute Otitis Media (AOM) Events With Coinciding Viral Infection in Children Followed From 2 to 24 Months of Age in the Finnish Otitis Media (FinOM) Studies

Type of AOM event	FinOM Cohort Study 759 acute otitis media events (rate per person year)	FinOM Vaccine Trial 1416 acute otitis media events (rate per person year)
Evaluable[a]	1.34	1.28
Coindicing with		
Any virus	0.72	0.87
Rhinovirus[b]	0.55	0.41
Enterovirus	NA	0.33
Respiratory syncytial virus	0.12	0.12
Influenza virus A	0.04	0.03
Adenovirus	0.04	0.03
Parainfluenza virus 3	0.03	0.05
Parainfluenza virus 2	0.005	0.003
Parainfluenza virus 1	0.002	0.003
Influenza virus B	0	0.01
Parechovirus	NA	0.01
2 or more viruses	0.06	0.13

Detection of a given virus in nasopharyngeal aspirate and/or middle ear fluid required for a positive score.
NA = not analyzed.
[a]Nasopharyngeal aspirate sample and at least one middle ear fluid sample per event available for virological analyses.
[b]The methods used for rhinovirus detection were different in the two studies.

TABLE III. Presence of Respiratory Viruses in Middle Ear Fluid (MEF) Samples and in Nasopharyngeal Aspirates (NPA) in 759 Acute Otitis Media Events of 329 Children Followed in the Finnish Otitis Media Cohort Study From 2 to 24 Months of Age

		Virus positive NPA specimen								
Virus positive MEF specimen	N	Rhinoviruses	RSV	Influenza virus A	Adenovirus	Parainfluenza virus 3	Parainfluenza virus 2	Parainfluenza virus 1	Influenza virus B	Any virus(es)
		223	64	19	18	17	3	1	—	322
Rhinovirus	197	**111**	3	—	9	2	—	—	—	117
RSV	47	4	**41**	2	1	—	2	—	—	42
Influenza virus A	9	1	2	**8**	—	—	—	—	—	8
Adenovirus	6	2	1	—	**4**	—	—	—	—	5
Parainfluenza virus 3	4	1	—	—	—	**4**	—	—	—	4
Parainfluenza virus 2	2	—	2	—	—	—	**2**	—	—	2
Parainfluenza virus 1	—	—	—	—	—	—	—	—	—	—
Influenza virus B	—	—	—	—	—	—	—	—	—	—
Any virus(es)	252	116	41	8	12	6	2	—	—	**163**

All numbers refer to events. Due to double infections, the same events may occur more than once in the table, and the numbers are therefore not simply additive. The row entitled N gives the numbers of events with any given virus in the nasopharyngeal aspirate and the column entitled N gives the number of events with any given virus in the middle ear fluid. Bolded numbers indicate the acute otitis media events, in which the same virus was detected concurrently in the nasopharyngeal aspirate and in at least one MEF sample. —, no event in this category.

(Table IV). RSV and rhinovirus were found concurrently in nasopharyngeal aspirate and middle ear fluid samples in 61 and 42% of the events positive for RSV and rhinovirus, respectively. The enterovirus probe yielded a concurrent positive result in 42% of the events (not searched for in the Cohort Study) (Table IV).

Seasonal Variation of Viral Findings

Monthly rates of acute otitis media events with coinciding viral infection showed similar seasonal variation in both studies (Fig. 1). The seasonal occurrence of virus positive acute otitis media events followed in general the incidence of acute otitis media events. The highest incidence rates of virus negative acute otitis media events were seen in November to December 1996 in the Cohort Study and in the Vaccine Trial in winter 1996–1997. The typical peaks of rhinovirus epidemics were seen in the autumn and in the spring in both studies. Enteroviruses (studied only in the Vaccine Trial) were present during most of the year including the winter and spring months, and annual RSV epidemics were also seen (Fig. 1). Influenza viruses A and B were present from January to March and parainfluenza viruses 3 were mainly detected in late spring. Adenoviruses were present almost throughout the year. Parechoviruses were detected from September to March.

DISCUSSION

In these two large prospective studies, we have shown that in children less than 2 years of age, a specific respiratory virus is present in the nasopharynx and/or middle ear fluid in two thirds of all acute otitis media events. It is likely that the true proportion of acute otitis media events with an associated virus infection is even higher as we did not test for all respiratory viruses [Nokso-Koivisto et al., 2000; Hirsilä et al., 2001; van den Hoogen et al., 2001] and the antigen detection test used is known to find only part of the corresponding infections [Räty et al., 2003]. Rhinoviruses were detected most frequently, but enteroviruses also appeared to be a major group among respiratory pathogens. For these two virus groups, a PCR method was used whereas RSV, the next virus in order, was detected only by antigen test. In earlier studies, it has been suggested that RSV is one of the most important viruses contributing to development of acute otitis media [Henderson et al., 1982; Heikkinen et al., 1999]. The prevalences of different virus groups were the same between the two studies. Likewise, the seasonal and age specific occurrence rates of different viruses were similar in both studies and in agreement with our previous preliminary report of the Cohort Study based on nasopharyngeal specimens only [Vesa et al., 2001].

Two different detection methods were used, RT-PCR for picornaviruses and TR-FIA for other viruses. The PCR method is generally considered the most sensitive detection method and therefore, the use of these two methods may have biased the relative proportions of

TABLE IV. Presence of Respiratory Viruses in Middle Ear Fluid (MEF) Samples and in Nasopharyngeal Aspirates (NPA) in 1,416 Acute Otitis Media Events of 611 Children Followed in the Finnish Otitis Media Vaccine Trial From 2 to 24 Months of Age

Virus positive MEF specimen	N	Virus positive NPA specimen										
		Rhinoviruses	Enteroviruses	RSV	Influenza virus A	Adenovirus	Parainfluenza virus 3	Parainfluenza virus 2	Parainfluenza virus 1	Influenza virus B	Parechovirus	Any virus(es)
		407	287	129	34	34	56	3	3	12	10	892
Rhinoviruses	236	**191**	10	3	1	7	4	—	—	—	—	205
Enteroviruses	226	44	**152**	10	2	5	2	1	1	—	1	202
RSV	89	11	7	**83**	—	—	1	—	—	—	—	84
Influenza A	16	1	2	—	**14**	—	—	—	—	—	—	14
Adenovirus	4	—	—	—	—	**4**	—	—	—	—	—	4
Parainfluenza 3	28	2	1	1	—	—	**26**	—	—	—	2	26
Parainfluenza 2	—	—	—	—	—	—	—	—	—	—	—	—
Parainfluenza 1	1	—	1	—	—	—	—	—	**1**	—	—	1
Influenza B	—	—	—	—	—	—	—	—	—	—	—	—
Parechovirus	3	—	1	—	—	—	—	—	—	—	—	1
Any virus(es)	579	240	168	91	15	15	29	1	2	—	3	**466**

All numbers refer to events. Due to double (or triple) infections the same events may occur more than once in the table, and the numbers are therefore not simply additive. The row entitled N gives the number of events with any given virus in the NPA specimen and the column entitled N gives the number of events with any given virus in the MEF sample. Bolded numbers indicate the acute otitis media events, in which the same virus was detected concurrently in the NPA and in at least one MEF. —, no event in this category.

different viruses. It has been shown that the PCR method might be more sensitive than antigen detection method for analysis of respiratory viruses, [Henkel et al., 1997; Räty et al., 1999] but even PCR method is not perfect. For example, in this study the primers used for RT-PCR do not cover all different rhinovirus strains [Blomqvist et al., 1999]. Varying epidemiology is another confounding factor in complicating the comparison of results from different studies. It was unexpected to note that the proportion of acute otitis media events coinciding with rhinovirus infections was smaller in the Vaccine Trial than in the Cohort Study even though the combined cell-culture-PCR method used in the latter study had a sensitivity of 66% only as compared to that of direct RT-PCR used in the Vaccine Trial [Blomqvist et al., 1999].

We have shown recently, by using the current multiplex RT-PCR method, either rhinovirus or enterovirus RNA in 29% of nasopharyngeal aspirate specimens from children without significant concurrent respiratory symptoms [Nokso-Koivisto et al., 2002a]. However, in most of these children the presence of viral RNA in the nasopharynx could be associated with a recent or forthcoming symptomatic infection, or exposure to infection in the household. Therefore, we believe that a great majority of the viruses detected in our acute otitis media patients are involved in the initiation of the respiratory infection observed.

It is likely that the prevalence of viral infections would have been even greater if the methods used had covered all the potential causative virus groups and strains. Although in the Vaccine Trial ten different respiratory viruses were tested for, there are still many known and probably also unknown viruses that may cause respiratory infections in children. Coronaviruses and influenza C viruses cause upper respiratory infections, but to a lesser degree in this age group [Nokso-Koivisto et al., 2000; Hirsilä et al., 2001]. We have previously reported results from a sample of specimens derived from the FinOM Cohort Study revealing that about 3% of tested nasopharyngeal aspirate specimens collected from the children presenting with symptoms of respiratory infection contained human coronavirus RNA [Nokso-Koivisto et al., 2000]. The recently discovered human metapneumoviruses have been reported to cause in children respiratory infections that clinically resemble RSV infections [van den Hoogen et al., 2001]. In both the Finnish Otitis Media Cohort Study and Vaccine Trial, the study of seasonal prevalence revealed a difference in winter 1996–1997 between all acute otitis media events and those associated with documented viral infections. One could speculate that this divergence reflects an epidemic caused by one of the known viruses not included in our virus detection panel, or an epidemic due to an unknown virus.

In the Vaccine Trial, enteroviruses appeared to be associated with one fourth of the acute otitis media events. In this study, rhino- and enterovirus infections were identified by first amplifying a part of the genome using primers shared by the two genera and subse-

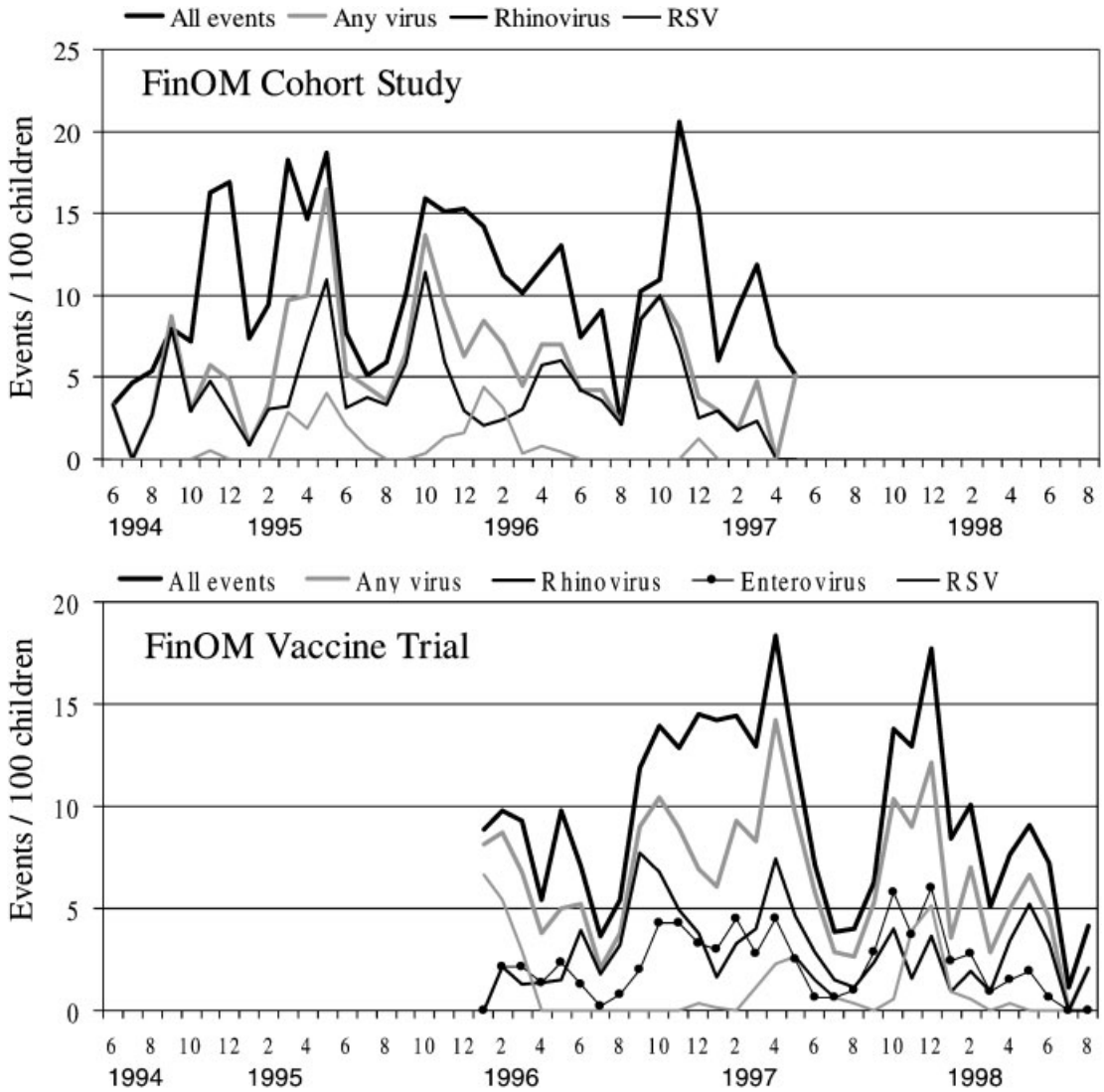

Fig. 1. Monthly occurrence of all evaluable acute otitis media events and occurrence of acute otitis media events positive for any virus, rhinoviruses, enteroviruses, and RSV in the Finnish Otitis Media Cohort Study and Vaccine Trial in children followed from 2 to 24 months of age.

quently differentiating them with the aid of virus group specific probes. Validation analysis of the method showed that the enterovirus probe also detects some rhinovirus prototypes. Although a sample was interpreted to be positive for enterovirus only if it was negative for the rhinovirus probe some of these samples may have contained aberrantly reacting rhinoviruses. Conventional identification of enteroviruses by virus culture and serotyping by neutralizing antibodies is difficult and time consuming. Better methods are desirable for reliable differentiation of enteroviruses from rhinoviruses and for easy identification of the relevant serotypes.

Previously, a specific viral diagnosis of upper respiratory infection or acute otitis media had little clinical relevance. However, therapy for virus infections is developing rapidly and efficient drugs exist for some viruses [Hayden, 2001; Meissner, 2001; Ison et al., 2002]. This poses new requirements for virus diagnostics and new opportunities to treat viral infections in selected target groups. For example, treatment of children with frequently recurring infections or prevention of acute otitis media under specific situations might be feasible in the future.

In conclusion, we have presented the virological findings associated with acute otitis media in children less than 2 years of age in two large prospective cohort studies. Respiratory viruses were present in two thirds of the acute otitis media events and, in half of the events studied, a picornavirus infection was detected. Use of PCR-based methods for the detection of the other respiratory viruses as well might have increased the overall virus detection rate in the studied samples. These results confirm the important role of viruses in the development of acute otitis media. In the future, the virus groups associated most commonly with acute otitis

media, i.e., picornaviruses and RSV, should be considered when aiming at prevention of acute otitis media in children.

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