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**Viral upper respiratory tract infections
in young children**

Academic dissertation

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of the Medical Faculty of the University of Helsinki,
in the Auditorium of the Department of Otorhinolaryngology,
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ABSTRACT

Background:

Viral upper respiratory infection is the most common reason for seeking medical care for children. Recurrent viral respiratory infections and subsequent complications (e.g. acute otitis media (AOM)) are an enormous burden for children, their families, and society. Etiologic studies of viral infections are becoming increasingly important, with the emergence of new antiviral drugs and vaccines.

Methods:

Two cohorts of children (n=329 and n=611) were followed from 2 to 24 months of age in two prospective studies (Finnish Otitis Media (FinOM) Cohort Study and FinOM Vaccine Trial). Nasopharyngeal aspirate and middle ear fluid specimens, and paired serum samples were collected and examined for common respiratory viruses. In both FinOM studies, the association of viral infections with AOM was investigated. A sample of specimens from the FinOM Cohort Study was analyzed for human coronavirus RNA using the RT-PCR method, and the viral etiology of frequently recurring respiratory infections (FRRIs) in children was examined. In addition, nasopharyngeal specimens were analyzed for viral RNA in another group of children without concurrent respiratory symptoms.

Results:

Respiratory viruses were detected in two-thirds of the AOM events, and in half of the events studied, a rhinovirus or enterovirus infection was present. The third most common virus was respiratory syncytial virus (RSV). A specific susceptibility to a defined type or group of viruses does not seem to be a major explanatory factor for FRRIs in young children. Coronavirus RNA was present in 2.4% of respiratory specimens of children less than two years of age. In asymptomatic children, picornavirus RNA was detected in 29% of nasopharyngeal specimens by the RT-PCR method, but 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.

Conclusions:

In children less than two years of age, rhinoviruses, enteroviruses, and RSV appear to cause most of the upper respiratory infections and are associated with AOM. In the future, these viruses should be considered when aiming at prevention and treatment of respiratory infections and AOM in young children.

TIIVISTELMÄ

Tausta:

Akuutit ylähengitystieinfektiot ovat ylivoimaisesti tärkein syy lasten lääkarissäkäynteihin. Jotkut lapsista sairastavat samoissakin olosuhteissa ylähengitystieinfektioita runsaammin kuin toiset. Lasten toistuvat virusperäiset infektiot ja niiden tavalliset komplikaatiot (esim. välikorvatulehdus) ovat taakka lapsille, vanhemmille ja yhteiskunnalle. Ylähengitystieinfektioiden etiologisten tutkimusten merkitys on korostunut entisestään kun uusia viruslääkkeitä on tulossa kliiniseen käyttöön.

Menetelmät:

Pääosa tutkimusten aineistosta liittyy Finnish Otitis Media (FinOM) kohorttitutkimukseen ja rokote-tutkimukseen (FinOM Cohort Study and Vaccine Trial). Näissä kahdessa prospektiivisessä tutkimuk-sessa seurattiin yhteensä 940 lasta 2 kuukauden ikäisestä aina kaksivuotiaaksi asti. Tutkimuksen aikana analysoitiin hengitystieinfektioita aiheuttavia viruksia nenäimulima-, välikorvaerite- ja seeru-minäytteistä. Molemmissa FinOM tutkimuksissa analysoitiin eri virusten esiintyvyys lasten äkillisis-sä korvatulehduksissa. FinOM kohorttitutkimuksessa tutkittiin RT-PCR menetelmällä koronavirus-ten esiintyvyyttä nenänielu- ja välikorvaeritenäytteissä sekä analysoitiin infektiokierteisten lasten ylähengitystieinfektioiden virusetiologiaa. Lisäksi RT-PCR menetelmällä tutkittiin respiratoristen virusten RNA:n esiintyvyyttä oireettomien lasten nenänielussa.

Tulokset:

Modernien analyysimenetelmien avulla osoitettiin, että äkillisen välikorvatulehduksen aikana alle kaksivuotiailla lapsilla jopa kahdella kolmasosalla on todettavissa virus joko nenänielu- tai välikor-vaeritteessä. Yleisimmät respiratoriset virukset korvatulehduksen aikana olivat rino- ja enteroviruk-set, joita oli osoitettavissa yli puolessa korvatulehduksista, sekä RSV. Infektiokierteisillä lapsilla ei ole erityistä riskiä sairastua tiettyjen virusten aiheuttamiin infektiioihin. RT-PCR menetelmällä ko-ronaviruksia oli osoitettavissa 2.4 prosentissa pienten lasten nenäimulima- ja välikorvaeritenäytteis-tä. Oireettomiltakin lapsilta on löydettävissä virusten RNA:ta nenänielusta, joten tutkimuslöydös on aina suhteutettava oirekuvaan

Johtopäätökset:

Alle kaksivuotiailla lapsilla rinovirukset, enterovirukset ja RSV aiheuttavat valtaosan ylähengitys-tieinfektioista ja välikorvatulehduksista. Nämä virukset tulisi ottaa huomioon suunniteltaessa lasten virusperäisten ylähengitystieinfektioiden ennaltaehkäisyä ja hoitoa.

CONTENTS

ABSTRACT	4
TIIVISTELMÄ	5
CONTENTS	6
ABBREVIATIONS	8
LIST OF ORIGINAL PAPERS	9
1 INTRODUCTION	11
2 REVIEW OF THE LITERATURE	13
2.1 Epidemiology of upper respiratory infections	13
2.2 Clinical entities and complications of viral respiratory infections in young children	14
2.2.1 Common colds	15
2.2.2 Acute otitis media	15
Respiratory viruses in acute otitis media	16
2.3 Frequently recurring respiratory infections in children	19
2.3.1 Risk factors	19
2.4 Viral etiology of upper respiratory infections	22
2.5 Specific features of respiratory virus families	25
2.5.1 Picornaviruses	25
Rhinoviruses	25
Enteroviruses	26
Parechoviruses	26
2.5.2 Respiratory syncytial viruses	27
2.5.3 Influenza viruses	27
2.5.4 Coronaviruses	28
2.5.5 Adenoviruses	29
2.5.6 Parainfluenza viruses	30
2.5.7 Metapneumovirus	30
3 AIMS OF THE STUDY	33

4	PATIENTS AND METHODS	35
4.1	Study design and patients	35
4.1.1	Finnish Otitis Media Cohort Study (I, II, III)	35
4.1.2	Finnish Otitis Media Vaccine Trial (I)	35
4.1.3	Presence of viral RNA in the nasopharynx of children (IV)	35
4.2	Ethics	36
4.3	Definitions	36
4.4	Sample collection	37
4.5	Combined culture-RT-PCR method for rhinoviruses	37
4.6	Reverse transcription PCR	37
4.7	Antigen detection	38
4.8	Serology	38
4.9	Statistical methods	38
5	RESULTS AND DISCUSSION	39
5.1	Viral etiology of upper respiratory infections (I-IV)	39
5.1.1	General aspects	39
5.1.2	Specific viral infections and acute respiratory diseases in young children	39
5.1.3	Methodological aspects	41
5.2	Presence of viral RNA in the nasopharynx of children without concurrent respiratory symptoms (IV)	43
5.3	Viruses associated with acute otitis media (I)	44
5.3.1	Concurrent virus detection in the nasopharynx and middle ear	46
5.3.2	Seasonal variation of viral findings in association with acute otitis media	47
5.4	Children with frequently recurring respiratory infections (III)	48
5.4.1	Viral etiology of frequently recurring respiratory infections	50
6	GENERAL DISCUSSION AND CONCLUSIONS	51
	ACKNOWLEDGMENTS	55
	REFERENCES	57

ABBREVIATIONS

Ag	Antigen detection
AOM	Acute otitis media
CF	Complement fixation
ELISA	Enzyme-linked immunosorbent assay
FinOM Studies	Finnish Otitis Media Studies
FRRRI	Frequently recurring respiratory infections
IF	Immunofluorescence
IFN- α	Interferon alpha
MEF	Middle ear fluid
NPA	Nasopharyngeal aspirate
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
RSV	Respiratory syncytial virus
TR-FIA	Time-resolved fluoroimmunoassay
URI	Upper respiratory infection

LIST OF ORIGINAL PAPERS

- I** Nokso-Koivisto J, Rätty R, Blomqvist S, Kleemola M, Syrjänen R, Pitkäranta A, Kilpi T, Hovi T. Presence of specific viruses in the middle ear fluids and respiratory secretions of young children with acute otitis media.
J Med Virol 72:241-248, 2004.
- II** Nokso-Koivisto J, Pitkäranta A, Blomqvist S, Kilpi T, Hovi T.
Respiratory coronavirus infections in children younger than two years of age.
Pediatr Infect Dis J 19:164-166, 2000.
- III** Nokso-Koivisto J, Pitkäranta A, Blomqvist S, Jokinen J, Kleemola M, Takala A, Kilpi T, Hovi T. Viral etiology of frequently recurring respiratory tract infections in children.
Clin Infect Dis 35:540-546, 2002.
- IV** Nokso-Koivisto J, Kinnari TJ, Lindahl P, Hovi T, Pitkäranta A. Human picornavirus and coronavirus RNA in nasopharynx of children without concurrent respiratory symptoms.
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1 INTRODUCTION

Acute upper respiratory infections (URI) are the most common acute diseases in children. Numerous long-term family and community studies have yielded abundant information about acute respiratory infections (Frost et al. 1932; McCammon 1971; Monto et al. 1974; Tupasi et al. 1990; Monto et al. 1993; Ray et al. 1993). These studies provide foundation for understanding the natural course and characteristics of respiratory infections.

Respiratory infections cause significant economic losses. In the United States, acute respiratory infections in 1998 resulted in an estimated 84 million visits to physicians; of which 25 million were due to upper respiratory infection and 13 million due to acute otitis media (AOM) (Gonzales et al. 2001). Half of the diagnoses among walk-in patients in a children's hospital have been associated with respiratory infections (Peltola 1982). Every fourth respiratory infection results in a visit to a

physician, with the proportion in infants rising to approximately one half of all respiratory infections (Monto et al. 1974). In children attending day-care centers in Finland, infectious diseases caused more than 90% of total costs of illness (deficient utilization of day-care centers, parents' lost working capacity, hospitalization, visits to physician, antibiotics) (Nurmi et al. 1991). Respiratory infections also cause productivity losses and absenteeism from work (Bramley et al. 2002).

Previously, viral diagnoses and etiologic studies of URI have had little clinical relevance. However, prevention and therapies for viral infections are developing rapidly, and antivirals for respiratory viruses other than influenza may be available in the near future (Hayden 2001; Ison et al. 2002; Heikkinen et al. 2003b). This poses new requirements for viral diagnostics, particularly rapid and easy detection of respiratory viruses.

2 REVIEW OF THE LITERATURE

2.1 EPIDEMIOLOGY OF UPPER RESPIRATORY INFECTIONS

The first large follow-up study of respiratory infections started some 80 years ago with approximately 10 000 university students and employees. The study reported the annual variation of respiratory infections, with typical high incidence in the winter and low incidence in the summer, and also indicated the association between mortality and the incidence of respiratory infections. In addition, minor respiratory infections with rhinitis as the main symptom were shown to have the highest incidence in the autumn, while influenza-like infections were mainly present in the spring (Frost et al. 1932).

In 1948-50, in a Cleveland study, the mean rate of respiratory infections was reported to be 8 infections per person-year in children less than 6 years of age (Badger et al. 1953). This annual respiratory infection rate is among the highest ever reported, possibly because of the frequent weekly visits by a study worker and the apparent eagerness of self-selected families to report even minor symptoms. Later, large community-based studies have reported that the mean annual number of acute respiratory infections is 5 in children less than 5 years of age and 3 in older children. With increasing age, the annual incidence of infections decreases, and adults have on average 1 to 2 URIs per year (McCammon 1971; Monto et al. 1993; Monto 2002). Children, especially boys, are at risk of developing URIs (McCammon 1971; Tupasi et al. 1990; Monto et al. 1993). A serologic analysis of over 58 000 patients revealed that most respiratory viruses are encountered in childhood and that a rapid increase in viral antibodies occurs in children until 6 to 10 years of age (Ukkonen et al. 1984). Community studies also established that interaction with other people spreads respiratory infections efficiently (Badger et al. 1953; Monto et al. 1974). A temporary

increase in incidence in the age group of 20 to 29 years has been reported, probably in connection with starting a family, and thus, increased exposure to infections through children. In line with this, the frequency of URIs is higher among females (Badger et al. 1953; Monto et al. 1974). The incidence of respiratory infections has been demonstrated to rise as family size increases to 5 persons per family (Badger et al. 1953), and especially among children, exposure to other children increases infection risk (Wald et al. 1988).

2.2 CLINICAL ENTITIES AND COMPLICATIONS OF VIRAL RESPIRATORY INFECTIONS IN YOUNG CHILDREN

Many clinical diseases have been shown to be associated with respiratory viruses (Table 1). In children, the most common viral respiratory infections are simple URIs (the common cold) and AOM. These entities are discussed in more detail in later sections. Other important aspects of viral infections in children are summarized briefly below.

Viruses are the most common cause of lower respiratory tract infections in children (Woensel et al. 2003), with respiratory syncytial virus (RSV), parainfluenza virus type 3, and influenza viruses occurring most frequently (Sunakorn et al. 1990; Ray et al. 1993; Yun et al. 1995; Andreoletti et al. 2000; Tsai et al. 2001; Weigl et al. 2001). In addition, rhinoviruses have been shown to cause pneumonia and bronchiolitis in infants and children (Juvé et al. 2000; Hayden 2004). The recently discovered human metapneumovirus has been reported to induce RSV-like respiratory infections (van den Hoogen et al. 2001), and this virus may also produce lower respiratory tract infections in children (Williams et al. 2004). Viral infections have been shown to trigger wheezing and

exacerbate asthma in children (Jennings et al. 1987; Mertsola et al. 1991; Johnston et al. 1995; Rawlinson et al. 2003). Virus-induced asthma exacerbations may be severe, and increase the need of hospitalisation (Gern et al. 2000). Acute laryngitis is a viral disease most frequently caused by parainfluenza viruses, and less frequently by RSV and influenza viruses (Gröndahl et al. 1999; Hall 2001a).

Over 40% of acute tonsillitis cases are associated with a respiratory virus, and in one-third a virus may be the sole pathogen. Adenovirus is the predominant cause of viral tonsillitis, with other common causative agents being Epstein-Barr viruses, influenza viruses and enteroviruses (Douglas et al. 1984; Putto 1987; Putto-Laurila et al. 1999; Tsai et al. 2001). Enteroviruses, especially coxsackievirus A24 and enterovirus 70, are known to cause acute hemorrhagic conjunctivitis, often in epidemics (Christopher et al. 1982; Shulman et al. 1997; Wairagkar et al. 1999). Conjunctivitis is also a common sign at the time of upper respiratory infection caused by adenovirus (Mitchell et al. 2000; Weigl et al. 2000).

Table 1. Respiratory tract infections and viral causative agents in children.

Disease	Adeno- viruses	Corona- viruses	Entero- viruses	Influenza viruses	Parainfluenza viruses	RSV ¹	Rhino- viruses
Common cold	+	++	++	++	+	+	+++
Tonsillitis	+++	-	++	+	+	+	-
Laryngitis	+	-	+	++	+++	+	+
Bronchitis	+	+	+	+++	++	+++	+
Bronchiolitis	+	+	+	++	++	+++	++
Pneumonia	+	+	+	+++	++	+++	++

¹ RSV = respiratory syncytial virus

Although viral respiratory infections are often considered relatively harmless, in developing countries they are an important cause of mortality, especially when followed by bacterial complications. In a study conducted in the Philippines, the respiratory tract infection-specific mortality rate (caused by virus and/or bacteria) was 8.9 per 1000 in children less than four years of age. In this study, a respiratory virus was associated with one-third of acute respiratory infections, but a bacterium was identified in only 10% of cases (Tupasi et al. 1990). In a study by Hong et al. (2001) the mortality rate was 6% among apparently healthy children who were hospitalized because of adenoviral lower respiratory tract infection. Even during the last decade, enterovirus outbreaks in the Far East have been associated with a high incidence of mortality (Chan et al. 2000; Lu et al. 2002).

2.2.1 Common colds

The common cold is mainly a viral disease. Mäkelä et al. (1998) found respiratory viruses to be associated with two-thirds of common cold events, while bacteria were cultured in only 4% of cases. Rhinoviruses are the leading cause of common colds (Arruda et al. 1997; Mäkelä et al. 1998; Monto et al. 2001), and during an epidemic, rhinoviruses may be associated with up to 80% of common colds (Arruda et al. 1997). Enteroviruses have previously rarely been sought in respiratory infection studies due to the lack of convenient diagnostic methods. However, after introduction of the reverse transcription polymerase chain reaction (RT-PCR) technique, it has become evident that enteroviruses are also frequently associated with common colds. In one recent study, every fourth URI in children was associated with an enterovirus (Ruohola et al. 2000). Although both RSV and influenza A virus are well known for causing lower respiratory infections, they also generate upper respiratory

infections (Vesa et al. 2001). Coronaviruses have been associated with common colds in children, and in one study as many as 30% of respiratory infections were coronavirus-associated (Isaacs et al. 1983). However, according to serologic studies, coronaviruses seem to be quite prevalent, but may produce a large number of subclinical infections (Macnaughton 1982).

In children, acute respiratory infections often spread into the paranasal sinuses, causing mucosal edema and accumulation of mucus. A recent study reported that 68% of children (mean age 5.7 years) with uncomplicated upper respiratory infections had major abnormalities in the paranasal sinuses, which mainly resolved after 2 weeks without antimicrobial treatment (Kristo et al. 2003). In another study, 70% of children with purulent rhinorrhea as the only symptom had opacification of the paranasal sinuses on computer tomography scans (Schwartz et al. 2001). Young children almost always have nasal secretions in paranasal sinuses during upper respiratory infections (so-called rhinosinusitis). Based on this, sinusitis in children is an extension of the common cold. However, evidence of direct viral infection of the maxillary sinus in children is lacking. In adults with acute maxillary sinusitis, a virus can be found in 10% of maxillary secretion samples by viral culture (Hamory et al. 1979) and in 40% by PCR (Pitkäranta et al. 1997). In addition, rhinovirus ribonucleic acid (RNA) has been found inside the epithelial cells of the maxillary sinus mucosa at the time of acute sinusitis by *in situ* hybridization (Pitkäranta et al. 2001).

2.2.2 Acute otitis media

AOM is one of the most common infectious diseases among children. In the Finnish Otitis Media (FinOM) Cohort Study, 62% of children had at least one AOM episode by the age of two years and 8% had six or more episodes (Kilpi et

al. 2001). In another study conducted in Finland, the incidence of AOM was estimated to be 32 per 100 person-years in children less than 10 years of age (Joki-Erkkilä et al. 1998). According to claims data from a large health insurer in the United States, among children less than 2 years, one-fourth had 6 or more office visits for management of otitis media per one year (Thompson et al. 1999). In children with respiratory infections, more than one-half of the prescribed antibiotics have been for AOM (Pichichero et al. 2000).

Respiratory viruses in acute otitis media

Viral respiratory infections have been shown to alter middle ear pressure, which in turn can cause accumulation of mucus and pathogenic agents in the middle ear. In a study with adults experimentally infected with influenza virus A, 59% of patients developed negative middle ear pressure and 15% of those infected developed middle ear effusion, which was negative for bacteria (Buchman et al. 1995). In a study by Arruda et al. (1997), every fifth adult with a common cold had abnormal middle ear pressure during the first days of rhinovirus infection. Transient negative middle ear pressure can occur in two-thirds of children with uncomplicated URI. Differences in age, detection of respiratory virus, or type of virus did not affect the development of abnormal middle ear pressure (Winther et al. 2002).

Viral infection has been suggested to cause prolonged symptoms of AOM. Respiratory viruses have been detected more often from the middle ear fluid (MEF) or nasopharyngeal aspirate (NPA), or both, in children with prolonged symptoms of AOM and failure of initial antimicrobial therapy (Arola et al. 1990b). Rhinovirus-positive culture in MEF has been associated with persistence of bacteria or occurrence of new bacteria in the MEF (Sung et

al. 1993). Controversial results have also been published reporting that the duration of symptoms was longer in children with no detected pathogens than in children with bacteria or virus in MEF (Chonmaitree et al. 1986).

Many studies during the past decades have shown the close relationship between viral infections and the development of AOM (Table 2). Several studies have also demonstrated an association between the outbreaks of viral infections and a simultaneous increase in AOM incidence (Henderson et al. 1982; Sarkkinen et al. 1985; Ruuskanen et al. 1989; Arola et al. 1990a). In a 14-year longitudinal study concurrent or previous respiratory infection was associated with one-fourth of AOM episodes, and the risk of AOM was increased especially after RSV, adenovirus, or influenza virus infection (Henderson et al. 1982).

Further evidence of viral otitis media has emerged with viruses being isolated by viral culture directly from MEF of patients with AOM. One of the first reports was from Yoshie in 1955, who isolated the influenza viruses from MEF samples of patients with AOM during an influenza epidemic (Yoshie 1955). Culture methods at that time were not very efficient; Grönroos et al. cultured 399 MEF samples, but failed to isolate a single virus (Grönroos et al. 1964). Somewhat later, RSVs were isolated from MEF samples during a RSV infection (Berglund et al. 1966; Grönroos et al. 1968). After development of viral culture techniques, the proportion of virus-positive AOM has risen. Chonmaitree et al. (1986) found 20% of MEFs to be virus-positive by cell culture.

As diagnostic methods have improved, the association between viruses and AOM has grown stronger (Heikkinen et al. 2003a). Antigen detection methods were developed, and they are faster and easier than viral culture techniques or serological analyses. However, respiratory viruses for which antigen detection was not available, e.g. rhinoviruses and enteroviruses,

Table 2. Selected data from studies of viruses associated with acute otitis media (AOM).

Study	No. of children	No. of MEF	Virus detection method ¹	Virus infection associated with AOM ² (%)	Proportion of viruspositive MEF (%)
Yoshie 1955	10	10	Culture, serology	40	40
Grönroos 1964	322	399	Culture	-	0
Berglund 1966	27	44	Culture, serology	37	33
Tilles 1967	90	NR	Culture, serology	27	3
Grönroos 1968	11	22	Culture, serology	100	55
Klein 1982	53	53	Ag	34	25
Chonmaitree 1986	84	84	Culture	39	20
Sarkkinen 1985	137	137	Ag	42	18
Arola 1990	88	88	Culture, Ag	48	19
Chonmaitree 1992	271	271	Culture, Ag, serology	46	22
Heikkinen 1999	456	815	Culture, Ag, serology	41	17
Pitkäranta 1998	92	92	RT-PCR	75	48
Chonmaitree 2000	40	65	Culture, PCR	NR	74

¹ Ag = antigen detection, RT = reverse transcription, PCR = polymerase chain reaction

² Specific virus detected in nasopharyngeal aspirate (NPA) and/or middle ear fluid (MEF) specimen(s), and/or a viral infection documented serologically from paired serum samples
NR = not reported

were not regularly analyzed. Combining different detection methods (culture, antigen detection, serology) respiratory viruses have been associated with up to one-half of AOM events (Klein et al. 1982; Sarkkinen et al. 1985; Arola et al. 1990a; Chonmaitree et al. 1992; Heikkinen et al. 1999). In studies using antigen detection, RSV has usually been the most common virus associated with AOM. Arola et al. (1990a) detected RSV in 15% of MEF samples during AOM, and in 7% RSV was the only pathogen found. RSV has been suggested to be one of the most potent viruses to cause AOM since it is most often detected concurrently in the NPA and MEF during AOM (Heikkinen

et al. 1999). In addition, among children hospitalized due to RSV infection, up to 75% were diagnosed as having AOM (Heikkinen et al. 1995). However, in many studies, rhinoviruses, the most common cause of URI in children, were either not sought or only viral culture was used (Klein et al. 1982; Heikkinen et al. 1999).

A significant step forward has been the development of the PCR method, which enables the detection of respiratory viruses, including those for which antigen detection tests are not suitable or are unavailable. PCR is usually more sensitive than viral culture and antigen detection methods, and has been developed for many

respiratory viruses (Gröndahl et al. 1999; Chonmaitree et al. 2000). By RT-PCR, respiratory viruses have been found to be associated with over 70% of AOM events (Pitkäranta et al. 1998; Chonmaitree et al. 2000).

Evidence of an association between viruses and AOM is also based on virus-specific intervention studies. One-third of AOMs occurring during influenza outbreaks can be prevented by immunizing children with influenza virus vaccine (Heikkinen et al. 1991) or by giving specific antiviral treatment at the time of influenza virus infection (Whitley et al. 2001). In addition, high doses of RSV-enriched immunoglobulin have been reported to prevent development of AOM in high-risk children (Simoes et al. 1996).

For a physician, it would be very beneficial to differentiate viral AOM from bacterial disease. However, thus far, viral and bacterial AOM cannot be separated from each other by clinical symptoms or signs. Only a bulging tympanic membrane has been associated with detection of bacteria or bacterial-viral combinations from the MEF during AOM (Arola et al. 1990a; McCormick et al. 2000).

2.3 FREQUENTLY RECURRING RESPIRATORY INFECTIONS IN CHILDREN

A certain group of children suffers from recurrent and prolonged respiratory tract infections for years. Although these diseases are often mild and self-limiting, the burden of “being sick all the time” can be unbearable for both the children and their parents. Defining frequently recurring respiratory infections (FRRI) is arbitrary, with many different descriptions being found in the literature. Beard et al. (1981) defined infection-prone children as those having 6 or more URIs per year. In other studies, FRRI has been defined as 4 to 12 URIs per year (Isaacs et al. 1982; Venuta et al. 1996; Chapelin et al. 1997; Giraudi et al. 1997; Hewson-Bower et al. 2001). In reality,

respiratory infection rate in children form a continuum without segregation into “infection-prone” and “normal” children.

2.3.1 Risk factors

The mystery of respiratory infection-prone children has puzzled medical personnel for a long time, and many risk factors have been studied (Table 3). The most important risk for respiratory infections is age; young children have the highest incidence of respiratory infections (McCammon 1971; Harsten et al. 1990; Monto et al. 1993;

Table 3. Suggested host and environmental risk factors for frequently recurring respiratory infections in children.

Host risk factor	Reference
Age	McCammon 1971, Harsten 1990, Monto 1993
Gender	Monto 1974, Kim 2000
Genetic susceptibility, family risk	Söderström 1991a, 1991b
Reduced immunocompetence	Beard 1981
Deficient production of interferon alpha	Isaacs 1981, Pitkäranta 1993, 1999
Mannose-binding lectin insufficiency	Koch 2001
Primary immunodeficiency	Rosen 1995
Ciliary dyskinesia	Chapelin 1997
Gastroesophageal reflux	Guill 1995
Psychosocial stress	Drummond 1997
Environmental risk factor	Reference
Siblings	Badger 1953, Tupasi 1990, Monto 2002
Day care	Alho 1990, Harsten 1990, Louhiala 1995, Celedon 1999, Nafstad 1999
Parental smoking	Bryd 1995, Peat 2001
Short duration of breast-feeding	Alho 1990, López-Alarcón 1997
Air pollution	Jaakkola 1991
Vitamin A deficiency	Pinnock 1986

Monto 2002). In addition, boys appear to be more prone to respiratory tract infections, especially during the first years of life (Badger et al. 1953; Monto et al. 1974; Kim et al. 2000). Major defects in immune responses are rare in children with FRRI (Rosen et al. 1995). Some other biological factors, such as ciliary dyskinesia, gastroesophageal reflux, or swallowing dysfunction with chronic aspiration, may cause recurrent infections, but these diseases are also rarely demonstrated (Guill 1995; Chapelin et al. 1997; Sheikh et al. 2001).

Minor deficiencies in such immune responses as reduced neutrophil chemotaxis and fungicidal capacity in infection-prone children have been reported (Beard et al. 1981). In addition, some reports suggest that infection-prone children may have a genetic susceptibility to infections. Children with mannose-binding lectin insufficiency have an increased risk for URI (Koch et al. 2001). A prospective twin and triplet study came to the conclusion that recurrent AOM might be due to a genetic property (Casselbrant et al. 1999). Children with FRRI also tend to come from families with general health problems, and the children have a susceptibility to infections for many years, from a very young to school age (Söderström et al. 1991a; Söderström et al. 1991b). Leukocyte cultures from children with FRRI have been reported to have a lowered capacity to produce interferon alpha (IFN- α) *in vitro* (Isaacs et al. 1981; Vanecek et al. 1985; Pitkäranta et al. 1993; Pitkäranta et al. 1999). It has been studied whether measurement of IFN- α production could be used to predict future recurrence of infections (Pitkäranta et al. 1999). However, the interindividual variation was too great for use for this purpose. Reduced production of IFN- α in infection-prone children led to the implication that these children could be susceptible to certain viruses or virus groups. In a study by Isaacs et al. (1982) viral infections of FRRI children were compared to those of unaffected siblings in the

same age group. No difference in the proportions of different viruses was isolated from index and control children (Isaacs et al. 1982).

Environmental factors, such as day care outside of the home (Wald et al. 1988; Alho et al. 1990; Harsten et al. 1990; Louhiala et al. 1995; Celedon et al. 1999; Nafstad et al. 1999), household smoking (Bryd et al. 1995; Peat et al. 2001), diet (Pinnock et al. 1986), and a polluted environment (Jaakkola et al. 1991), have been associated with FRRI. In addition, working mothers with child-care arranged outside the home have been suggested to seek for medical care for minor symptoms more easily than other mothers (Horwitz et al. 1993). Crowded conditions increase the risk; a child with many siblings is at higher risk of getting respiratory infections (Badger et al. 1953; Tupasi et al. 1990; Monto 2002). Especially among children less than 2 years of age, attending day-care centres is a risk factor for infections, while family care seems to pose a minor risk (Harsten et al. 1990; Louhiala et al. 1995). Indeed, Paradise and coworkers reported a strong association between otitis media and the degree of exposure to other children, whether at home or at a day-care center (Paradise et al. 1997).

Short-duration or lack of breast-feeding is often mentioned as a risk factor for FRRI (Alho et al. 1990; Arifeen et al. 2001). In one study, receiving only breast-milk was reported to reduce acute respiratory infections during the first four months of life, but thereafter the reduction was not significant (López-Alarcón et al. 1997). By contrast, in a recent study in Belarus, breast-feeding did not seem to reduce respiratory infections (Kramer et al. 2001). Overall, breast-feeding is nevertheless considered to be an important factor in preventing infections and infant mortality in developing countries (Arifeen et al. 2001).

Psychosocial stress has been reported to be elevated in children with FRRI and to be associated with lower levels of secretory

immunoglobulin A and its ratio to albumin in saliva as indicators of deficient local mucosal immunity (Drummond et al. 1997). On the other hand, as the authors suggested, psychological disorders might be due to recurrent infections. It has been also suggested that psychological treatment will shorten the duration of respiratory symptoms and increase the secretory IgA concentration in children suffering from FRRIs (Hewson-Bower et al. 2001).

2.4 VIRAL ETIOLOGY OF UPPER RESPIRATORY INFECTIONS

In the 1960s, the development of virus detection methods made it possible to analyze most of the known respiratory viruses or virus groups by cell culture or serologic methods. Since then, many studies have focused on the association between respiratory viruses and different respiratory tract infections. When comparing the data from various studies, it is important to realize that the spectrum of etiologic agents may vary between studies because of differences in study settings, sample types or microbiological detection methods. For example, the viruses that cause severe infections may be more dominant in hospital-based studies than in community-based studies. In addition, many studies search for only a limited number of causative pathogens.

A comparison of different studies on acute respiratory infections and viral findings in children is shown in Table 4. Over the years, the reported proportion of virus-positive respiratory infections has increased due to improved detection methods. In a community study covering 11 years, viruses were detected in 22% of respiratory infections using cell culture of nasal and throat swabs (Monto et al. 1993). In one study exploiting a variety of detection methods, the virus-positivity rate in young adults was 69% (Mäkelä et al. 1998). An even higher positivity rate has been observed in a study of children; 86% of nasopharyngeal samples were virus-positive at the time of upper respiratory infection using an antigen detection method and RT-PCR (Ruohola et al. 2000). The reported prevalence of rhinoviruses in different studies of respiratory infections has increased after introduction of PCR, a more sensitive detection method.

According to several studies, the prevalence of different viruses in respiratory infections may vary between different age groups. In young children, common viruses are reported to be rhinoviruses (Monto et al. 1993;

Ruohola et al. 2000; Vesa et al. 2001), RSV (Tupasi et al. 1990; Kim et al. 2000; Manjarrez et al. 2003), and parainfluenza viruses (Monto et al. 1993; Weigl et al. 2000), as well as enteroviruses in some studies (Hazlett et al. 1988; Tsai et al. 2001). Older children and teenagers often have influenza and rhinovirus infections, and in adults rhinoviruses are again at the forefront (Monto et al. 1974; Monto et al. 1993).

Although virus-positivity and the proportion of different viruses seem to be fairly similar worldwide, the environment and the location of the study may also affect results. In developed countries, rhinoviruses and RSV appear to be the most common viral agents to cause respiratory infections in children (Weigl et al. 2000; Vesa et al. 2001).

Many viruses have typical annual seasonality (Monto et al. 1993; Lina et al. 1996; Kim et al. 2000). In addition, when comparing data between different studies, it is important to consider that the incidence of specific viruses can vary greatly between years (Kim et al. 2000). For example, RSV epidemics in Finland have been shown to occur in 2-year cycles (Waris 1991), and coronavirus OC43 epidemics have been reported to occur 2 to 4 years apart (Kaye et al. 1971).

The use of PCR methods as a diagnostic tool for respiratory infections is increasing. PCR methods are more sensitive than traditional virus detection methods (Henkel et al. 1997; Hyypiä et al. 1998; Avellon et al. 2001), and this has raised the question of the clinical relevance of virus-positive PCR findings. Very little is known about the presence of respiratory viruses in the nasopharynx of healthy children. Serologic studies have shown that asymptomatic viral infections do occur (Kaye et al. 1971). In addition, after natural or experimental rhinovirus infection, shedding of the rhinovirus in the

Table 4. Representative studies of etiology of respiratory tract infections in children.

Study	Study period	Type of study/ Country	No. of children	Age (y)	Virus detection method(s) ¹	Proportion (%) of virus-positive respiratory infections	Viruses ³						
							RSV	HRV	PIV	Infl	Ad	HEV	Proportion (%) or rate of viruses associated with respiratory infection event ²
Cooney 1972	1965-69	Community/ USA	302 person-years	<9	Culture	2.24 rate per person-year	-	0.86	-	0.38	0.53	0.46	
Monto 1993	1965-71	Community/ USA	539 person-years	<4	Culture	31	0.29	0.60	0.28	0.15	0.18	-	
Hazlett 1988	1976-81	Hospital/ Kenya	822	<5	Culture, Ag	41	12	7	2	1.1	2	20	
Jain 1991	1981-82	Hospital/ India	736	<5	Culture, Ag	22	5	-	5	6	3	-	
Arruda 1991	1984-86	Community/ Brazil	175	<5	Culture, Ag	35	0	17	6	2	4	6	
Tupasi 1990	1985-87	Community/ Philippines	311	<5	Culture, Ag	33	13	-	5	2	4	5	
Lina 1996	1994-95	Community/ France	554 samples	<14	Culture, Ag	38	16	1.6	-	12	4	-	
Manjarez 2003	1995-96	Outpatient/ Mexico	179	<5	Culture, IF	49	18	-	8	13	9	-	
Weigl 2000	1995-99	Hospital/ Germany	1281	<16	RT-PCR	33	13	-	3	8	8	4	
Kim 2000	1996-98	Hospital/ Korea	1048	<11	Culture	23	8	-	4	7.5	5	-	
Ruohola 2000	1996-98	Outpatient/ Finland	194	0.7-3.9	Ag, RT-PCR	86	4	38 ⁴	3.6	2.5	1.5	25 ⁴	
Tsai 2001	1997-99	Outpatient and hospital/Taiwan	6986	<12	Culture, IF	32	2	-	2	9	4	13	

¹ Ag = Antigen detection, IF = immunofluorescence, RT-PCR = reverse transcription polymerase chain reaction

² Proportion of all respiratory infections in the corresponding study, except in studies by Cooney et al 1972 and Monto et al 1993 the numbers indicate rate per person-year.

³ Ad = Adenoviruses, Infl = Influenza viruses, HEV = Human enteroviruses, HRV = Human rhinoviruses, PIV = Paramfluenza viruses, RSV = Respiratory syncytial viruses

⁴ In addition, 16% of infections were positive for unclassified picornaviruses.

nasopharynx may continue for up to 3 weeks after onset of infection (Hendley et al. 1988; Arruda et al. 1997).

The problem of interpreting virus-positive findings was also an issue before. In the Seattle Virus Watch in 1965-69, over 200 individuals were followed for 2 years. Rates of viral cultures from nasopharyngeal swabs revealed that the number of cultured viruses increased a few days before the onset of an illness and remained high during the subsequent week. In this large study, viruses could be cultured from 3% of samples from persons who did not have respiratory symptoms 3 weeks prior to and 3 weeks after sample collection (Cooney et al. 1972).

By culture, viruses have been isolated from 13% to 27% of healthy (Isaia et al. 1985; Manjarrez et al. 2003) and from 3% of asthmatic (Horn et al. 1979; Jennings et al. 1987) children without concurrent respiratory symptoms. By using a combination of different detection methods, 47% of asymptomatic children had viruses in nasal wash samples, while over 80% of symptomatic, wheezing children were virus-positive (Rakes et al. 1999). Only a few studies exist in which the presence of viral RNA in the nasopharynx has been examined. In one of these (Johnston et al. 1993), picornaviruses were detected by RT-PCR method in 12% of asymptomatic children; only 1.4% of the children had been positive by viral culture. At the time of respiratory infection, 50% of the episodes were PCR-positive and 16% were culture-positive. Of children with asymptomatic chronic asthma, rhinovirus RNA was detected from the nasopharynx in 17% (Rawlinson et al. 2003). However, in another study of asymptomatic asthmatic children, over 80% virus-positivity has been reported, while 5% of swabs from healthy controls were virus-positive (Marin et al. 2000). All asthmatic children had received long-term inhaled glucocorticoids, which may affect the high virus-positivity rate. In most of these studies, a 2- to 4-week

symptomless period was required prior to sample collection, but later symptoms were not registered. Therefore, findings may partly be due to future illness.

2.5 SPECIFIC FEATURES OF RESPIRATORY VIRUS FAMILIES

2.5.1 Picornaviruses

The family of Picornaviruses is a group of small, nonenveloped viruses with single-stranded positive-sense RNA genome. The group is divided into the following 9 genera: entero-, hepato-, kobu-, parecho-, rhino-, aphto-, cardio-, erbo-, and teschoviruses (<http://www.iah.bbsrc.ac.uk/virus/Picornaviridae>; King et al. 2000). In terms of causing respiratory illnesses to humans, the most important viruses are rhino-, entero-, and parechoviruses. These three human picornaviruses are discussed in detail in the text below.

Rhinoviruses

Rhinoviruses are the largest group of picornaviruses, including 101 different serotypes. All but 10 of these serotypes use intercellular adhesion molecule-1 as a receptor. Rhinoviruses are the predominant cause of the common cold all over the world and in all age groups. Rhinoviruses occur relatively more often in children than in adults (Monto et al. 1993). At the age of 2 years, 91% of children have antibodies against rhinoviruses and 79% of children have experienced culture- or RT-PCR-confirmed rhinovirus infection (Blomqvist et al. 2002a). Human rhinovirus infections typically occur in the early fall and in the spring (Mäkelä et al. 1998; Vesa et al. 2001). The dominant transmission route remains unclear (Goldmann 2000). The optimal temperature for growth is 33°C to 35°C *in vitro*, and the nose and nasopharynx are the primary sites for replication. The infectious dose for humans is very small and the infection proceeds rapidly, with the incubation period being as short as 8 to 12 hours. Inflammatory responses of the host tend to prolong symptoms of infection even after virus replication has diminished (Hendley 1999).

Usually rhinovirus infection results in a mild illness characterized by a runny and stuffy nose, a sore throat, and coughing and hoarseness (Lina et al. 1996; Arruda et al. 1997; Monto et al. 2001; Gwaltney 2002), but more severe symptoms resembling influenza may also occur (Boivin et al. 2002). Although rhinoviruses are generally thought to cause only mild upper respiratory infections, they are also associated with acute lower respiratory tract infections, wheezing, bronchiolitis, and pneumonia in children (Chidekel et al. 1997; Kim et al. 1998; Hayden 2004). This association has been strengthened by culturing rhinoviruses directly from tracheal aspirates in children (Schmidt et al. 1991) and by detecting rhinovirus RNA by *in situ* hybridization from bronchial biopsies (Papadopoulos et al. 2000). In addition, rhinoviruses often cause exacerbations of pre-existing airways diseases, such as asthma or chronic obstructive pulmonary disease (Hayden 2004).

Due to its many different serotypes, developing a vaccine for rhinoviruses is unlikely. Treatment of rhinovirus disease is also problematic because infection often proceeds quickly and medication should be started as soon as the first symptoms occur or shortly thereafter. Many different treatment methods have been investigated. Intranasal interferon treatment has been shown to be as effective as prophylaxis for rhinovirus infections, but side-effects limit the use (Hayden et al. 1986; Monto et al. 1986b). A capsid-binding agent, pleconaril, also effective against enteroviruses, has been the most promising drug to date and has demonstrated that developing a treatment for the main causes of the common cold is possible (Hayden et al. 2003). Unfortunately, since further studies revealed that pleconaril induces hepatic

cytochrome P-450 3A enzymes and may have potential drug interactions, regulatory approval was not granted for the oral formulation of the drug. According to the review by Savolainen et al., an intranasal formulation of the drug is, however, currently under investigation (Savolainen et al. 2003). Other drugs studied include intranasally administered receptor decoy soluble intercellular adhesion molecule-1 (tremacamra), 3C protease inhibitors rupintrivir and pyridone, and oral anti-picornavirus capsid-binder pirodavir (Turner et al. 1999; Hayden 2001; Ison et al. 2002; Hayden et al. 2003). However, none of these medications is thus far in clinical use, and therefore further studies on the prevention and treatment of rhinovirus infections are needed.

Enteroviruses

Enteroviruses are a large group of picornaviruses, and at least 64 immunologically distinct serotypes are known to cause infections in humans: polioviruses (3 serotypes), coxsackie viruses group A (23 serotypes), and group B (6 serotypes), echoviruses (28 serotypes) and enteroviruses 68-71 (4 serotypes). A new classification divides human enterovirus serotypes into 5 different species partly based on the genome organization and sequence similarity: polioviruses and human enteroviruses A-D (King et al. 2000; Pallansch et al. 2001). Recombination is a significant and relatively frequent mechanism in the evolution of enteroviruses (Santti et al. 1999). Nonpolio enteroviruses are very common worldwide, and most primary infections of different serotypes occur in childhood. Enterovirus infections are reported to be more prevalent in highly populated areas and under poor hygienic conditions (Pallansch et al. 2001). A study covering a 20-year period in Finland revealed that the most common enteroviruses isolated from clinical specimens were coxsackie viruses A9, B3, and

B5 and echoviruses 11, 30, and 22 (reclassified as human parechovirus 1), but the predominant enteroviruses in sewage were coxsackie viruses B2-5 and echoviruses 6 and 11 (Hovi et al. 1996). In this study, differences in virulence might explain the divergence in prevalence between clinical and sewage isolates. The distribution of different serotypes in the Finnish clinical specimens differed to some extent from those in other countries. A 14-year surveillance of more than 20 000 enterovirus isolates from the United States showed that the 15 most common enterovirus types accounted for 81% of all isolates (Strikas et al. 1986). Some enteroviruses cause distinct epidemics (mainly echoviruses), while others occur with varying frequency but never present a clear epidemic (Strikas et al. 1986).

Enteroviruses are transmitted probably by hand contact and autoinoculation to the mouth, nose, or eyes. Especially in areas with poor sanitary conditions, fecal-oral transmission predominates. From 50% to 90% of nonpolio enterovirus infections are suggested to be subclinical, and if they produce clinical illness it is usually a self-limited, nonfocal, febrile illness. The virus usually has an incubation period of 7 to 14 days. (Pallansch et al. 2001)

Pleconaril, which is discussed in more detail in the section on rhinoviruses, is also effective against enterovirus infections (Hayden et al. 2003). However, commercially available drugs against enteroviruses are lacking.

Parechoviruses

Human parechoviruses are a recently established group of viruses. Parechoviruses 1 and 2 were formerly classified in the enterovirus genus and known as echoviruses 22 and 23. However, based on molecular and biological properties, they have been separated into their own genus (Hyypiä et al. 1992; Stanway et al. 1994; Ghazi et al. 1998; Oberste et al. 1998).

Antibodies against parechovirus 1 increase rapidly with age; 89% of children have antibodies at the age of 1 to 2 years, and almost all adults are seropositive (Joki-Korpela et al. 1998). An analysis of clinical samples over a 20-year period revealed that parechovirus 1 was very common in the Finnish population (Hovi et al. 1996). Parechovirus infections occur year-round, mainly affecting young children (Joki-Korpela et al. 2001).

2.5.2 Respiratory syncytial viruses

Human RSV is an enveloped, single-stranded negative-sense RNA virus with two distinct serotypes, types A and B. RSV belongs to the genus *Pneumovirus* within the family of *Paramyxoviridae*.

RSV infections are present in all age groups, but they predominate in children and especially in infants. Up to 70% of infants have been reported to be infected with RSV during the first year of life, and the rest are infected by the age of 2 years (Walsh et al. 1999). In a Finnish study, RSV seropositivity reached 80% by the age of 6 years (Ukkonen et al. 1984). Reinfections by RSV are common (Hall 2001a). Typically, RSV epidemics occur during the winter months, sometimes starting in the late fall and continuing until early spring (Monto et al. 1993; Yun et al. 1995; Lina et al. 1996; Weigl et al. 2001). In Finland, RSV epidemics occur every 2 years, and during the epidemic year 2 separate epidemic peaks can be seen (Waris 1991). The pattern of 2-year epidemics is different from that in, for example, France (Freymuth et al. 1991).

RSV infections are transmitted by direct contact with infectious secretions and inoculated in nasal mucosa or the eye. The virus replicates in human nasal and bronchial epithelial cells with an incubation period of approximately 5 days (Walsh et al. 1999). Typical symptoms are

nasal congestion, coryza, fever, and productive cough (Lina et al. 1996; Hall et al. 2001b). RSV may cause severe diseases especially in young children. In a study by Monto et al. (1993), RSV infections resulted in a consultation to physician even more often than infections caused by influenza viruses or bacteria. RSV is the leading cause of bronchiolitis and acute wheezing in young children (Hall 2001a).

Ribavirin has been used as a specific antiviral treatment for RSV infections. It is recommended as a possible treatment only for a selected group of infants at high risk for serious RSV disease (Walsh et al. 1999). Intravenous, enriched RSV immunoglobulin can be used as prophylaxis for RSV infections in high-risk children or neonates (Meissner et al. 1996). Prophylactic treatment with palivizumab, a humanized RSV monoclonal antibody, during the epidemic season has been reported to be associated with a decreased rate of hospitalization for RSV lower respiratory tract infections (The IMPact-RSV Study Group 1998). VP14637 is a new anti-RSV fusion inhibitor that is much more effective than ribavirin in *in vitro* tests (Ison et al. 2002). Candidate vaccines against RSV infections are under development in clinical studies (Polack et al. 2004).

2.5.3 Influenza viruses

Influenza viruses are enveloped RNA viruses with three types: A, B, and C. Influenza A virus is the most important and can be further subtyped according to the composition of the envelope proteins (hemagglutinin and neuraminidase). The natural hosts for influenza A viruses are aquatic birds and various mammals, such as humans, pigs, and horses. In a rare event, two different influenza A viruses, possibly from two different host species, can infect a single host. By reassortment of the segmented genomes, a new

virus with unpredictable pathogenicity can be created (antigenic shift), which may result in a pandemic. Annual epidemics of influenza A and B viruses are caused by antigenic drift, with the genes encoding the surface antigens undergoing stepwise mutation. Eventually, the change in antigens results in a variant capability to cause illnesses, and antibodies from previous infections do not provide protection against this new virus strain. Because of rapid drift, a new composition of influenza vaccine must be developed every year. Influenza B infections occur mainly in humans. Influenza C is mostly a human virus, although it has been isolated from pigs as well. Influenza C viruses are difficult to culture and isolate, and therefore, knowledge of this virus is limited. The RT-PCR method has made research of influenza C viruses easier (Hirsilä et al. 2001).

Most data about influenza viruses are for types A and B because, due to detection difficulties, influenza C viruses have not been included in the studies. Transmission of influenza viruses occurs mainly by inhalation of small-particle aerosols or by direct contact. Viruses are rapidly spread to the trachea, bronchi, and lower airways. Most often influenza virus infections are associated with a characteristic clinical picture, including cough, high fever, pharyngitis, myalgia, and headache (Lina et al. 1996; Gwaltney 2002). Influenza type A and B cause infections that can range from asymptomatic infections and common colds to serious illnesses with systemic complications, such as pneumonia (Mäkelä et al. 1998; Zambon 1999). Even in healthy adults, influenza infection may last 1 to 2 weeks, and among elderly and immunocompromised patients the disease can be lethal (Zambon 1999). Influenza virus epidemics are associated with excessive mortality (Simonsen et al. 1997). Influenza A and B infections typically occur in a seasonal pattern and in winter epidemics, which have variable intensities (Monto 1994; Yun et al. 1995). Influenza virus C has been reported to

occur in winter and spring, and to be associated with 3.5% of common cold in adults analyzed by RT-PCR and serologic methods (Hirsilä et al. 2001).

Amantadine and the related drug rimantadine can be used for treatment of influenza A virus infections. The newer group of drugs are neuraminidase inhibitors zanamivir and oseltamivir, which are effective against influenza viruses A and B (Meissner 2001). Both inactivated and live attenuated vaccines have been developed for influenza viruses (Treanor 1999), but in annual vaccinations inactivated vaccines are used. The intranasally administered influenza vaccine contains live attenuated, cold-adapted influenza A and B viruses, and the U.S. Food and Drug Administration has recently approved the vaccine to be marketed in the United States (http://www.fda.gov/fdac/features/2003/503_flu.html).

2.5.4 Coronaviruses

Human coronaviruses are large enveloped RNA viruses. There are three groups of coronaviruses, but only two of them, group I including strain 229E and group II including strain OC43, are known to cause diseases in humans.

The diagnosis of coronaviruses has previously been laborious because viral culture is difficult. The epidemiology and clinical picture of these diseases have been based mainly on serologic studies. The use of the antigen detection method (Hierholzer et al. 1994) has been limited, and RT-PCR was only developed relatively recently (Myint et al. 1994).

According to serologic studies, human coronaviruses seem to be common in populations, producing a large number of subclinical infections (Macnaughton 1982). Clinical expression has mainly been associated with mild upper respiratory infections (Kaye et al. 1971; Isaacs et al. 1983; Macnaughton et al.

1983). Children less than 2 years of age have been reported to have practically no antibodies against coronavirus OC43, but a rapid increase in antibody prevalence occurs after the age of 2 years. By age 6, almost all children are seropositive, depending somewhat on the serologic test used (Hovi et al. 1979). In an earlier study, more than one-half of children were seropositive by the age of 5 years, and among adults approximately 70% had antibodies against coronavirus type OC43 and 31% against type 229E (McIntosh et al. 1970). Coronaviruses may be present in some degree throughout the year, but larger epidemics occur 2 to 4 years apart (McIntosh et al. 1970; Macnaughton 1982; Isaacs et al. 1983; Gill et al. 1994; Lina et al. 1996). Coronaviruses have an incubation period of approximately 5 days. During infection the major symptoms and signs are sore throat, cough, coryza, fever, pharyngitis, and cervical adenitis (Kaye et al. 1971; Isaacs et al. 1983; Lina et al. 1996). No treatment or prevention exists for coronavirus infections. Interferons and several proteinase inhibitors have been studied for treatment of infections, but none of these are in clinical use (Denison 1999).

In November 2002, the first cases of a mysterious, severe pneumonia, later to become known as severe acute respiratory syndrome (SARS), occurred in the province of Guangdong in southern China. This illness had such respiratory infection symptoms as cough, fever, and breathing difficulty and was characterized by very high mortality (Cyranoski 2003; Rosling et al. 2003). Only half a year later, the etiologic agent of SARS was described to be a novel coronavirus (Drosten et al. 2003; Ksiazek et al. 2003). This epidemic showed how effectively air travel and globalization can spread infectious diseases. SARS affected over 8000 patients and caused 774 deaths in 26 countries on 5 continents (Peiris et al. 2003). This disease can also occur in children (Bitnun et al. 2003; Hon et al. 2003), with the youngest patient being a 56-day-old

premature infant (Sit et al. 2003). SARS seems to be less aggressive in young children; the clinical course is milder and shorter and mortality is lower than in adults (Bitnun et al. 2003; Hon et al. 2003). After efficient protocols were instigated to prevent spread of the disease, the worldwide SARS epidemic faded rapidly, and on July 2003 the World Health Organization reported that the last human chain of transmission had been broken (Peiris et al. 2003).

2.5.5 Adenoviruses

Adenoviruses are nonenveloped DNA viruses with 49 different serotypes in humans. Most adenoviruses are considered to be respiratory pathogens, although diseases involving the gastrointestinal tract, urinary bladder, liver, pancreas, and central nervous system have also been described. Adenovirus types 40 and 41 are known to cause only gastroenteritis. Serotypes 3 and 7 have been reported to be associated with epidemics and more severe diseases (Mitchell et al. 2000; Hong et al. 2001; Ryan et al. 2002). Adenoviruses infrequently cause common colds, and respiratory infections caused by these viruses tend to be severe, characterised by high and prolonged fever and strong inflammatory response (Lina et al. 1996; Kawasaki et al. 2002). Necrotizing bronchitis or fatal diseases also may occur, albeit rarely (Mitchell et al. 2000; Hong et al. 2001). By the age of 3 years, 60-70% of children appear to be seropositive for adenoviruses (Ukkonen et al. 1984). Adenoviruses are present year-round but occur most frequently in the winter months (Monto 1994; Yun et al. 1995; Lina et al. 1996). Although community-acquired epidemics have been described (Mitchell 2000), adenoviruses more often cause occasional epidemics in semi-closed communities, such as garrisons or orphanages (Ryan et al. 2002).

Adenoviruses are usually transmitted by the fecal-oral route or by aerosols. Infection often begins in the eyes, nasopharynx, or lungs and then spreads to other organs. Cells may also become persistently infected, resulting in viral shedding years after infection. During respiratory infections caused by adenoviruses the major symptoms and signs are rhinitis, nasal congestion, fever, cough, conjunctivitis, and tonsillitis (Lina et al. 1996; Mitchell et al. 2000; Weigl et al. 2000; Tsai et al. 2001).

Live, enteric-coated adenovirus type 4 and 7 vaccines have been developed and used especially in the US army since 1971. However, in 1995, the sole manufacturer of these vaccines ceased production, and thus these vaccines are no longer available (Ray et al. 1993). No antiviral treatment against adenovirus infections exists.

2.5.6 Parainfluenza viruses

Parainfluenza viruses are single-stranded, enveloped RNA viruses that belong to the family *Paramyxoviridae*. There are four serotypes, with types 1-3 being clinically important respiratory pathogens. Parainfluenza viruses, especially type 3, have been described as causing respiratory infections in children of all ages (Knott et al. 1994; Reed et al. 1997; Ruohola et al. 2000; Tsai et al. 2001). In one study, 10% of children aged less than 5 years had at least one parainfluenza virus 3 infection (Reed et al. 1997). Parainfluenza viruses can cause a broad spectrum of respiratory diseases, ranging from mild upper respiratory infections to pneumonia, but are most often associated with laryngitis (Knott et al. 1994). Parainfluenza viruses type 1 and 2 have been associated with laryngitis and upper respiratory infections in the age group 2 to 5 years, while type 3 seemed to mostly cause only mild respiratory infections in infants (Knott et al. 1994). Parainfluenza viruses can be endemic or epidemic in the autumn or spring (Monto et al.

1993; Yun et al. 1995; Wright 1999). They usually replicate in the nasopharyngeal epithelium, and the incubation period is 2 to 8 days. The viruses are transmitted mainly by direct contact or by large-particle aerosols (Hall 2001a).

No antiviral drugs are available for parainfluenza virus infections. Live attenuated human parainfluenza virus 3 vaccine has been studied, and in a clinical trial, it has appeared to be safe and efficient in children (Karron et al. 2003).

2.5.7 Metapneumovirus

Human metapneumovirus is a recently discovered respiratory virus. Van den Hoogen et al. (2001) reported the culture of a new viral isolate that showed a morphology resembling paramyxovirus. This virus was identified as a new member of the *Metapneumovirus* genus based on sequence homology and given the name human metapneumovirus.

Human metapneumovirus infections have been identified in patients in Europe, North America, Asia, and Australia (van den Hoogen et al. 2001; Howe 2002; Jartti et al. 2002; Peiris et al. 2003a; Esper et al. 2004). Metapneumovirus infections occur mainly between winter and early spring (van den Hoogen et al. 2001; Jartti et al. 2002). Serologic studies have revealed that by the age of 5 to 10 years almost all children have antibodies against human metapneumovirus, and 100% seroprevalence has been shown in adults (van den Hoogen et al. 2001; Ebihara et al. 2003). By RT-PCR, metapneumovirus RNA has been detected in children with community-acquired respiratory infection (Stockton et al. 2002) and in children hospitalized because of acute respiratory infection (Boivin et al. 2003; Maggi et al. 2003; Peiris et al. 2003a). In addition, in Finland, 9% of children with acute wheezing have been associated with human metapneumoviruses (Jartti et al. 2002).

The clinical symptoms caused by metapneumovirus are reported to be similar to those due to RSV, ranging from mild URI to severe lower respiratory tract infection with high fever, cough, sore throat, myalgia, and vomiting in all age groups (van den Hoogen et al. 2001; Stockton et al. 2002; Boivin et al. 2003; Viazov et al. 2003; Peiris et al. 2003a; Williams et al. 2004).

3 AIMS OF THE STUDY

This study was designed on the framework of the Finnish Otitis Media (FinOM) Studies, including the Cohort Study targeting risk factors of acute otitis media and the Vaccine Trial designed to evaluate of the efficacy and safety of pneumococcal conjugate vaccines. These studies gave a unique opportunity to explore viral etiology of upper respiratory infections by using a large collection of clinical samples and modern detection techniques. The purpose of the present

study was to investigate the association of specific virus infections with acute otitis media and upper respiratory infections in young children. A special emphasis is on children with recurrent respiratory infections to assess whether these children are prone to certain viruses or virus groups. Because a RT-PCR method was used for some virus analyses, another goal was to determine whether viral RNA could be detected from symptomless children.

Specific questions for which answers were sought were the following:

1. What is the association between specific viruses and acute otitis media in young children?
2. How common is human coronavirus in young children?
3. Do respiratory infection-prone children suffer more from certain virus infections than other children?
4. How often is picornavirus or coronavirus RNA present in the nasopharynx of children without concurrent respiratory symptoms?

4 PATIENTS AND METHODS

4.1 STUDY DESIGN AND PATIENTS

4.1.1 Finnish Otitis Media Cohort Study (I, II, III)

Altogether 329 children were enrolled to the FinOM Cohort Study, which was a prospective study to evaluate epidemiology and risk factors of AOM in young children. All infants born or living in the Hervanta area, in Tampere, were eligible to participate in the study if they were 2 months \pm 2 weeks old, had no prior immunisation with a pneumococcal vaccine, and their mothers could communicate fluently in Finnish. The children were enrolled in the study at 2 months of age and followed up until they were 24 months old. A special study clinic was established with 1 to 2 study doctors and 2 to 3 study nurses. The study clinic was open in the daytime on weekdays and also 3 hours per day on weekends and national holidays. Interview data were collected during 10 scheduled visits. The families were encouraged to take the child to the study clinic whenever symptoms of acute URI appeared, and especially if an AOM was suspected. During these sick visits the patient's history was recorded and a physical examination, including pneumatic otoscopy, was performed by the study physician. A nasopharyngeal aspirate (NPA) was obtained and in the case of AOM, myringotomy was performed and middle ear fluid (MEF) specimens were aspirated from the inflamed ear(s). In addition, if AOM was diagnosed, paired serum samples were obtained (Table 5).

4.1.2 Finnish Otitis Media Vaccine Trial (I)

FinOM Vaccine Trial was a prospective, randomized, and double-blind cohort study designed to evaluate of the efficacy and safety of two different sevenvalent pneumococcal

conjugate vaccines (Eskola et al. 2001; Kilpi et al. 2003). Altogether 2497 children were enrolled in the study, but only specimens collected from a sample of 611 children were subjected to virological analysis. The sample was randomly selected from 1610 children recruited during the first year of the trial. All children attended the study clinic at 2 months of age and seven times thereafter, during which interview data were collected. The study design was otherwise similar to the FinOM Cohort Study (Table 5).

4.1.3 Presence of viral RNA in the nasopharynx of children (IV)

The study population comprised 107 children who had been admitted for elective surgery to the Helsinki University Central Hospital. Fifty-three consecutive children were referred to the Department of Otorhinolaryngology for surgery because of upper respiratory tract problems and 54 to the Department of Ophthalmology for elective eye surgery. The study design and sample collection are described in detail in Study IV and Table 5.

Table 5. General characteristics of the studies.

Study	No. of children	Type of samples analyzed	Study period	Female
FinOM Cohort Study (Studies I, II, III)	329 (53% of the source population)	During URI: Nasopharyngeal aspirate (NPA) During AOM: NPA, middle ear fluid (MEF), paired serum samples	April 1994 - July 1997	171 (52%)
FinOM Vaccine Trial (Study I)	611 (random sample of 1610 children, 55% of the source population)	During AOM: NPA, MEF	December 1995 - September 1998	295 (48%)
Presence of viral RNA in the nasopharynx (Study IV)	107	NPA	November 1999 - March 2000	48 (45%)

FinOM = Finnish Otitis Media; URI = upper respiratory infection; AOM = acute otitis media

4.2 ETHICS

In all studies, written informed consent was obtained from the parents of study children. The FinOM Cohort Study and Vaccine Trial study protocols and consent forms were approved by the Ethics Committees of the National Public Health Institute, Tampere Health Center, and Tampere University Hospital. In Study IV, the research protocol was approved by the Ethics Committee of Helsinki University Central Hospital. Although myringotomy is no longer routinely used in the treatment of AOM (Puhakka et al. 1999), at the time the studies were planned and conducted, it was still a recommended diagnostic and therapeutic tool (Karma et al. 1987).

4.3 DEFINITIONS

URI: Symptoms and/or signs of an acute respiratory tract infection (rhinorrhea, fever, cough) and a diagnosis of acute respiratory infection assigned by the study doctor.

AOM: A visually abnormal tympanic membrane (with regard to color, position, and/or mobility) suggesting 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.

AOM event: An occasion on which AOM was diagnosed and both the NPA sample and at least one MEF sample were available for virological analyses.

AOM episode: A 30-day period commencing with a diagnosis of AOM and including possible sick visits. A new episode could start when at least 30 days had elapsed since the beginning of the previous episode.

URI episode: A 30-day period starting with any visit when the child was recorded as having URI and/or AOM and a NPA and/or a MEF specimen was obtained. A new episode could start when at least 30 days had elapsed since the beginning of the previous episode.

Frequently recurring respiratory infections (FRR): At least 9 URI episodes and/or at least 4 AOM episodes up to 24 months of age.

4.4 SAMPLE COLLECTION

In the FinOM Studies, all NPA samples were obtained for virological analysis using a sterile pediatric mucus extractor (Uno Plast A/S, Hundested, Denmark). In the FinOM Cohort Study, a small part of the NPA sample was taken for bacterial culture. In the case of AOM, after mechanical cleaning of the external ear canal, the tympanic membrane was anesthetized with 90% liquefied phenol. A myringotomy was performed on the anteroinferior part of the tympanic membrane, and MEF specimens were aspirated with a sterile glass suction tip. All MEF samples were rinsed from the collector with 0.7 or 1 ml of PBS-Lithium and divided for bacterial and virological analyses.

In the nasopharyngeal carriage study (IV), 1 ml of sterile physiological saline was administered to the nose, and nasopharyngeal aspirate samples were obtained with a sterile

suction catheter (Juhn Tym-Tap Middle Ear Fluid Collector). The catheter was inserted through a nostril to a depth of 4 to 8 cm, followed by gentle application of suction with an electric suction device. The nasopharyngeal aspirate was collected automatically in a small tube attached to the suction catheter.

The NPA and MEF samples were frozen immediately after collection and stored at -70°C for 2 months to 4 years before analysis. Sera were frozen immediately after collection and stored at -20°C.

4.5 COMBINED CULTURE-RT-PCR METHOD FOR RHINOVIRUSES

In the FinOM Cohort Study, rhinoviruses were detected by using a method that combined inoculation of a microwell culture of HeLa cells and subsequent RT-PCR (Blomqvist et al. 1999). Detection results based on acid sensitivity of the viral strains and the subsequent RT-PCR were combined. For the first 77 specimens, the harvested cultures were not, however, stored and only the culture result was available.

4.6 REVERSE TRANSCRIPTION PCR

NPA and MEF specimens were analyzed for corona-, rhino-, entero-, and parechovirus RNA using the RT-PCR method. Extraction of viral RNA from samples was performed with a commercial RNA isolation procedure (RNeasy®, Qiagen, GmbH, Heidelberg, Germany). Reverse transcription was carried out in 96-well plates including RNA and specific reverse primer(s). PCR was performed in 96-well plates with complementary DNA and specific forward primer(s). Pairs of primers complementary to the human coronavirus nucleocapsid protein gene for both human coronavirus OC43 and 229E were used in the same reactions. Similarly, in

picornavirus RT-PCR, a primer pair for rhinoviruses and enteroviruses, and a primer pair for parechoviruses were used in the same reactions. For rhino- and enteroviruses, the reverse primer was 5' –GAAACACGGACA CCCAAAGTA and the bionitylated forward primer was 5' –TCCTCCGGCCCCTGAATG, and the probe to detect rhinoviruses was 5' –AGGGTTAAGGTTAGCC. All of the other primers and probes used in the studies are described in the original articles.

In all studies, the microplate hybridization assay was carried out as described by Blomqvist et al. (1999). In Studies II and III, the PCR products were detected by immunoperoxidase reaction. In Studies I and IV, the PCR products were detected by a microplate hybridization method with specific lanthanide-labelled probes. At each step, several positive and negative controls were included. Interpretation of results is explained in the original studies.

4.7 ANTIGEN DETECTION

A one-incubation time-resolved fluoroimmunoassay (TR-FIA) was used for detection of antigens of adenoviruses, influenza viruses A and B, parainfluenza viruses 1, 2, and 3, and RSV. The crucial reagents were monoclonal antibodies (provided by the Department of Virology, University of Turku, Finland) with activity directed against specific epitopes of the structural proteins of the various viruses. The method takes advantage of a simultaneous incubation of the patient sample and the Europium-labelled detector antibody in wells, which have been coated with the respective capture antibody. Coating with the capture antibody, postcoating, and washing of the polystyrene microstrips (Labsystems Oy, Helsinki, Finland) were performed as described elsewhere (Halonen et al. 1983; Hierholzer et al. 1987; Hierholzer et al. 1989; Hierholzer et al. 1994).

4.8 SEROLOGY

A viral infection was documented serologically when a significant increase occurred in the concentration of antibodies to a given virus from serum I to serum II. Serum I was collected at the onset of the URI episode, and the convalescent phase serum sample (serum II) was obtained 4 weeks (range 14 to 42 days) later. A standard micromethod for the complement fixation (CF) test was used to detect viral antibodies to adenoviruses, parainfluenza virus types 1, 2, and 3, influenza viruses A and B, and coxsackievirus B-5 (Casey et al. 1965). An in-house complement fixation method for human rhinoviruses was carried out as described by Blomqvist et al. (2002a). Enzyme-linked immunosorbent assay (ELISA) was performed to detect antibodies against coronaviruses OC43 and 229E as described elsewhere (Mäkelä et al. 1998). The IgG antibodies against RSV were measured by a one-dilution ELISA as described by Rätty et al. (2004).

4.9 STATISTICAL METHODS

Relative frequencies of demographic and clinical characteristics between children with FRRI and non-FRRI groups were compared by using the chi-square test. The odds for virus-specific upper respiratory infections between non-FRRI children with at least one URI episode and children with FRRI were compared by using logistic regression analysis with the grouping variable as the only covariant in the model (Study III). The frequencies of virus-positive samples between children with infection-related diagnosis and those with non infection-related diagnoses were compared by using the chi-square test (Study IV).

5 RESULTS AND DISCUSSION

5.1 VIRAL ETIOLOGY OF UPPER RESPIRATORY INFECTIONS (I-IV)

5.1.1 General aspects

In the FinOM Studies, altogether 940 children were analyzed for viral infections, all 329 children in the Cohort Study and a random sample of 611 children in the Vaccine Trial. Overall compliance in the FinOM Cohort Study was rather good; 97% of the children were in the study at the age of 6 months, 94% at 12 months, 89% at 18 months, and 85% completed the follow-up. The median age at the time of drop-out was 15 months. In the FinOM Vaccine Trial, compliance was even better; only 10 (2%) of the random sample of 611 children did not complete the study.

Many studies over the decades have described the etiologic agents of respiratory infections in children. However, both FinOM Studies were large, well-organized, prospective cohort studies where viral diagnoses were done with modern, sensitive detection methods. Both studies can be considered population-based, and thus, the results represent viral infections in young children well. All children were carefully followed at the regular healthy visits in addition to sick visits. Regular samples for microbiological analysis were collected during the visits, and in case of AOM middle ear fluid samples were taken from each child. According to these studies, picornaviruses, particularly rhinoviruses, are the most common respiratory viruses in children less than 2 years of age. These studies were among the first to use modern technology in searching for picornaviruses in a large clinical material. Rhinoviruses and enteroviruses cannot be detected by antigen methods, which are known to be less sensitive than PCR methods. Had PCR methods been used for other respiratory viruses as well, the overall virus detection rate would likely have been even higher.

5.1.2 Specific viral infections and acute respiratory diseases in young children

In the FinOM Cohort Study, 287 of the 329 children (87%) had at least one URI episode during the study period. Altogether these children had 1358 URI episodes (median 4, range 0 to 13 episodes), 787 (58%) of which were positive for a respiratory virus. A URI episode was defined to be virus-positive if at the onset of the episode a specific virus was detected in the NPA and/or MEF specimen(s), and/or a viral infection was documented serologically from paired serum samples. Rhinoviruses were the most common viruses associated with respiratory infections, followed by RSV, parainfluenza viruses, and influenza viruses. Virological findings and rates of URI episodes are presented in Table 6.

The rate of URI episodes with concurrent virus detection in the FinOM Cohort Study was rather high compared with other studies of children (Tupasi et al. 1990; Arruda et al. 1991; Jain et al. 1991; Weigl et al. 2000). The proportion of virus-positivity can vary between studies depending on many different factors. The type of sample analyzed is one important factor; throat and nasal swab samples yield lower virus loads than do nasopharyngeal aspirate samples (Covalciuc et al. 1999; Heikkinen et al. 2002). The detection method chosen may affect results, and virus-positivity of URI in children may rise from 25% (viral culture only) to 86% (antigen detection and RT-PCR) (Monto et al. 1993; Ruohola et al. 2000). In studies with hospitalized children, the proportion of viruses that cause more severe diseases (e.g. RSV in infants) may be higher than among outpatient children (Tsai et al. 2001). Although studies of URI in children often last several years, varying epidemiology

Table 6. Association of specific virus infections with upper respiratory infection (URI) episodes in Finnish Otitis Media Cohort Study among 287 children with at least one URI episode.

Virus	Detection method episodes	No. of URI episodes (%)	Proportion ¹ of URI episodes	Rate of URI per person-year
Rhinoviruses	Culture-RT-PCR, serology	568	41.8	1.00
Respiratory syncytial viruses	Ag ² , serology	147	10.8	0.26
Parainfluenza viruses ³	Ag, serology	87	6.4	0.15
<i>Parainfluenza virus 1</i>		21		
<i>Parainfluenza virus 2</i>		7		
<i>Parainfluenza virus 3</i>		66		
Influenza viruses	Ag, serology	67	4.9	0.12
<i>Influenza virus A</i>		56		
<i>Influenza virus B</i>		11		
Adenoviruses	Ag, serology	63	4.6	0.11
Coronaviruses ⁴	Serology	21	1.5	0.04
<i>Coronavirus OC43</i>		15		
<i>Coronavirus 229E</i>		6		
Coxsackie B-5 virus ⁴	Serology	3	0.2	0.01
Two or more viruses	All methods	150	11.0	0.27
Virus-positive URI episodes	All methods	787	58.0	1.39
All URI episodes		1358		2.40

¹ Of all URI episodes

² Ag = Antigen detection

³ Seven URI episodes were positive for both parainfluenza viruses 1 and 3.

⁴ Coronaviruses and coxsackie B-5 viruses were detected by serology, analyzed at the time of AOM only. The coxsackie BV-5 antigen is likely to detect virus infections due to several other enterovirus serotypes as well.

can be a confounding factor in comparisons between different studies. Incidence of specific viruses can vary in consecutive years (Kaye et al. 1971; Waris 1991; Kim et al. 2000).

Rhinoviruses were by far the most common viruses detected in the FinOM Cohort

Study. One could argue that a sensitive detection method might overemphasize the proportion of rhinoviruses. However, overall, these findings are in line with other studies on respiratory infections in children (Arruda et al. 1991; Monto et al. 1993; Lina et al. 1996; Ruohola et al. 2000;

Weigl et al. 2000). Rhinoviruses have been reported to occur relatively more often in children than in adults (Monto et al. 1993), and over 90% of children at 2 years of age have antibodies against rhinoviruses (Blomqvist et al. 2002a). RSV was the second most common virus to cause URI in the FinOM Cohort Study. Although RSV is reported to be the most important cause of lower respiratory tract infections, especially bronchiolitis, it also causes upper respiratory infections in children (Lina et al. 1996; Andreoletti et al. 2000; Weigl et al. 2001; Manjarrez et al. 2003). It has been reported that influenza viruses are very common in young infants and are often detected in children requiring hospitalization (Munoz 2003). In our study, influenza virus A infections were responsible for only 4% of all URI episodes. Similarly, in a large community-based study with children influenza virus infections were relatively uncommon (Monto et al. 1993).

In the FinOM Cohort Study, 2.4% of a large amount of NPA specimens taken at the time of respiratory infection were coronavirus-positive by RT-PCR (Study II). In addition, coronaviruses were associated with 1.5% of AOM episodes by serology. The coronavirus infection rate here was lower than that found in some previous studies with children (Isaacs et al. 1983; Pitkäranta et al. 1998), possibly because children in the FinOM studies were very young. Several serologic studies suggest that the frequency of coronavirus infections in young children is relatively low, whereas in older children and adults, these infections are more common (McIntosh et al. 1970; Kaye et al. 1971; McIntosh et al. 1974; Hovi et al. 1979; Ukkonen et al. 1984). In the study by Kaye et al. coronavirus OC43 epidemics occurred irregularly (2 to 4 years apart), and during the epidemic up to 19% of respiratory infections were associated with a coronavirus (Kaye et al. 1971). Hence absence of a coronavirus epidemic might be the cause of differences in the

incidences of coronavirus infections between the FinOM studies and other studies. A similar conclusion can be made from seroprevalence studies in Finland (Hovi et al. 1979; Ukkonen et al. 1984). In addition, according to serologic studies almost one-half of the coronavirus infections may be asymptomatic (Kaye et al. 1971). However, knowledge of coronavirus infections is still very limited.

5.1.3 Methodological aspects

All MEF samples were flushed from the suction tip into phosphate-buffered saline and divided for bacterial and virological analysis. While rinsing will dilute MEF samples and might reduce the detection rate of viruses, it is often required to extent the small amounts of MEF samples for different analyses. The NPA and MEF samples were frozen immediately after collection and stored at -70°C for 1 to 3 years before analysis. Although this low temperature should preserve viruses well, it is possible that some viruses die during the long storage period. However, because most detection methods in these studies do not require a viable virus, the storage period should not have a large impact on the results. In Study III, serological diagnosis was attempted only in patients with AOM, and therefore, the relative proportion of virus-positivity is smaller for the viruses detected by serology only (coronaviruses, coxsackie viruses).

Four different detection methods were used in various combinations in our studies; combined culture with RT-PCR identification for rhinoviruses (Studies I, III), RT-PCR for picornaviruses and coronaviruses (Studies I, II, IV), antigen detection method (Studies I, III) for analysis of NPA and MEF samples, and serology (Study III). The use of different detection methods may have introduced bias into the relative proportions of different viruses. The PCR method may be more sensitive than viral

culture and antigen detection method for analysis of respiratory viruses (Johnston et al. 1993; Henkel et al. 1997; Avellon et al. 2001), but even this method is not infallible. For example, the primers used for RT-PCR (Studies I, III, IV) do not cover all different rhinovirus strains. However, for some respiratory viruses, no other fast and sensitive detection method exists (Ieven et al. 1997). Viral culture is laborious and time-consuming, and many diagnostic laboratories in Finland have abandoned this method as a primary diagnostic tool for respiratory infections. Currently, antigen detection is the most common method used for respiratory viruses from clinical samples in Finland. However, this method can be used only for those viruses for which suitable antibodies are available. Although serology is seldom used as a diagnostic method for common respiratory infections, epidemiological studies gain value if serology is included. In a subset of the FinOM Studies, serology was shown to increase the number of virus-positive findings considerably when combined with antigen detection methods (Räty et al. 2004). All detection methods have advantages and disadvantages, and it is difficult to single out one method as the best. It is reasonable to conclude that the more methods are combined, the higher (and the more reliable) the virus-positivity of respiratory infections.

In Studies I and IV, rhinovirus and enterovirus infections were identified by first amplifying a part of the genome using primers shared by the two genera and then differentiating them with the aid of virus group-specific probes. 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 rhino- and enteroviruses by viral culture and serotyping by neutralizing antibodies is difficult and time-consuming. Better methods are needed for reliable differentiation of enteroviruses from

rhinoviruses. It has recently become more evident that the border between rhino- and enteroviruses is very fuzzy. For example, human rhinovirus 87 and enterovirus 68 have been demonstrated to be genetically and antigenically highly similar and to represent the same serotype (Blomqvist et al. 2002b). In fact, joining the human enteroviruses and rhinoviruses in to a single Enterhinovirus genus has been proposed (unofficial proposal discussed within the Picornavirus Study Group of the International Committee for the Taxonomy of Viruses; T. Hovi, personal communication). However, an alternative approach can be used to report RT-PCR results: when the detected picornaviruses cannot be resolved into enteroviruses or rhinoviruses by the methods in use, the viruses could be regarded simply as unclassified entero- or rhinoviruses, perhaps later to be renamed enterhinoviruses (Ruohola et al. 2000).

5.2 PRESENCE OF VIRAL RNA IN THE NASOPHARYNX OF CHILDREN WITHOUT CONCURRENT RESPIRATORY SYMPTOMS (IV)

PCR methods are often believed to be almost “too sensitive”, and with their increasing use in viral diagnostics, the question arises that what is the relevance of a PCR-positive finding? Could viral RNA found in the nose or nasopharynx be a remnant of a past infection rather than evidence for etiology of a current infection.

In Study IV, 29% of NPA samples from children without concurrent respiratory symptoms were positive for viral RNA, 18% being positive for rhinoviruses and 11% for enteroviruses. Children with a diagnosis unrelated to infections had significantly less virus-positive samples than children with an infection-related diagnosis. Approximately two-thirds of children had had some respiratory symptoms in the four weeks and one-third in one week before sample collection. Only 4 of the 31 virus-positive samples were from children without an infection-related diagnosis or recent past or immediate future respiratory symptoms.

It is well known that bacterial colonization of the upper respiratory tract occurs in children soon after birth (Faden et al. 1997). Much less is known about the presence of viruses in the upper respiratory tract. Respiratory viruses have been detected from the nasopharynx in up to 47% of apparently healthy individuals (Cooney et al. 1972; Isaia et al. 1985; Johnston et al. 1993; Rakes et al. 1999; Manjarrez et al. 2003). In most of these studies, a symptomless period of 2 to 4 weeks prior to sample collection was required, but symptoms or signs after the sample was taken were rarely registered. Overall, viral RNA multiplied by RT-PCR can be found more often than infectious viruses in the nasopharynx of asymptomatic individuals, and patients with respiratory symptoms have much higher virus detection rates than asymptomatic children or adults (Johnston et al. 1993).

The findings in Study IV are in line with other studies, confirming that infectious viruses, antigens or nucleic acids can be found in the nasopharynx of asymptomatic individuals. The samples were collected during winter, which may in part explain the high occurrence of enteroviruses and rhinoviruses. Positive PCR findings in asymptomatic persons might represent subclinical infections, remnants of past infections or even signs of forthcoming infections. According to serological studies, viral infections often appear to be subclinical (Kaye et al. 1971; Macnaughton 1982; Pallansch et al. 2001). How long respiratory viruses can persist in the upper respiratory tract after acute infection remains to be determined. In natural and experimental rhinovirus infections in adults, viral shedding has been shown to continue at low levels for 2 to 3 weeks (Hendley et al. 1988; Arruda et al. 1997). Whether this is also true in children is unknown. It has been suggested that adults shed fewer viruses than children (Monto 1994). Detection of human rhino- and enterovirus RNA by RT-PCR (or by any method) in the nasopharynx must be interpreted cautiously because their presence alone does not establish causality of the concurrent illness. As always in clinical practice, laboratory findings have to be judged in relation to the symptoms and signs of the patient. However, in Study IV viral RNA detection was shown to be linked in most cases to past or future respiratory symptoms. Only four of the 31 children with virus-positive specimen did not have respiratory symptoms during the preceding 4 weeks or the forthcoming 2 weeks, or respiratory symptoms in family members. Therefore, the vast majority of the viruses detected at the time of URI can be assumed to be involved in the generation of the observed respiratory symptoms.

5.3 VIRUSES ASSOCIATED WITH ACUTE OTITIS MEDIA (I)

In the FinOM Studies, 940 children were followed and examined for evidence of viral respiratory infection. At the time of AOM, 2339 NPA and 3210 MEF specimens were available for virological analysis. In the FinOM Cohort Study, 42% of NPA samples and 33% of MEF samples, and in the FinOM Vaccine Trial 63% of NPA and 41% of MEF samples were positive for at least one analyzed respiratory virus during AOM. Altogether, respiratory viruses were associated with approximately two-thirds of evaluable AOM events in these two studies (Table 7). As many studies during the past decades have shown, viral infection is an important

predisposing factor for development of AOM, and viruses are significantly associated with AOMs (Tilles et al. 1967; Henderson et al. 1982; Sarkkinen et al. 1985; Chonmaitree et al. 1992; Pitkäranta et al. 1998; Heikkinen et al. 1999)

Since inclusion on of questionable cases of AOM could have biased the results, only AOM events that were confirmed by myringotomy and collection of MEF were included in the analysis. The proportion of children with at least one AOM was higher in the study group of the Vaccine Trial (75%) than in that of Cohort Study (62%). It is difficult to say why the proportions were different; in the Vaccine Trial, the families

Table 7. Overall virology results 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 (% ¹)	FinOM Vaccine Trial n (% ¹)	Combined results ² (% ¹)
Study group for virological studies	329	611	940
Children with at least one evaluable AOM event ³	203 (62% ⁴)	459 (75% ⁴)	662 (70% ⁴)
Evaluable AOM events (rate/person-year)	759 (1.34)	1416 (1.28)	2175 (1.30)
No. of AOM events with virus-positive NPA sample	322 (42%)	892 (63%)	1214 (56%)
No. of AOM events with virus-positive MEF sample	252 (33%)	579 (41%)	831 (38%)
Two or more viruses	34 (4.5%)	142 (10%)	176 (8%)
Virus-positive AOM events	406 (53%)	955 (67%)	1361 (63%)

¹ Proportion of all evaluable AOM events in the corresponding study/category unless otherwise indicated

² Some of the virus detection methods used were different in the two studies

³ Both nasopharyngeal aspirate (NPA) and at least one middle ear fluid (MEF) sample available for virus detection

⁴ Proportion of the corresponding study group

may have been more motivated to participate because of the benefits of receiving extra vaccines. The rates of AOM events were highly similar in both studies (Table 7).

In both FinOM Studies, rhinoviruses were detected most frequently, followed by enteroviruses (searched for only in the Vaccine Trial) and RSV. In the Vaccine Trial, picornaviruses (i.e. rhinovirus or enterovirus) were found to be associated with more than half of the AOM events, and a positive result was obtained with the enterovirus probe alone for 25% of events. The proportions of specific viruses associated with AOM are presented in Table 8.

For picornaviruses, a PCR method was used, whereas RSV, the next most common virus, was detected by antigen test, which is considered to be a less sensitive detection method. It is likely that the true proportion of AOM events associated with viral infections could be even higher, as we did not test for all respiratory viruses (e.g. coronaviruses, influenza virus type C, human metapneumovirus were not included), and the antigen detection test used is known to find only a portion of the corresponding infections (Räty et al. 2004). The use of different virus detection methods affects the results obtained. In studies using antigen detection methods, for instance, the

Table 8. Acute otitis media (AOM) events¹ with concurrent viral infection² in children followed from 2 to 24 months of age in the Finnish Otitis Media (FinOM) Studies.

AOM event coinciding with	FinOM Cohort Study 759 AOM events (% ³)	FinOM Vaccine Trial 1416 AOM events (% ³)
Any virus	53.5	67.4
Rhinoviruses ⁴	40.7	32.0
Enteroviruses	NA	25.4
Respiratory syncytial viruses	9.2	9.5
Parainfluenza viruses	2.7	4.5
<i>Parainfluenza virus 1</i>	0.1	0.2
<i>Parainfluenza virus 2</i>	0.4	0.2
<i>Parainfluenza virus 3</i>	2.2	4.1
Influenza viruses	2.6	3.3
<i>Influenza virus A</i>	2.6	2.5
<i>Influenza virus B</i>	0	0.8
Adenoviruses	2.6	2.4
Parechoviruses	NA	0.9
Two or more viruses	4.5	10.0

¹ Nasopharyngeal aspirate sample and at least one middle ear fluid sample per event available for virological analyses

² Detection of a given virus in nasopharyngeal aspirate and/or middle ear fluid required for a positive score

³ Proportion of all events in the category

⁴ Methods used for rhinovirus detection were different in the two studies

NA = not analyzed

role of RSV is emphasized (Klein et al. 1982; Heikkinen et al. 1999). In a study with RT-PCR methods, rhinoviruses were reported to be more common than RSV during AOM (Pitkäranta et al. 1998). As virus detection methods have developed during the past decades, the reported virus-positivity of MEF samples at the time of AOM has increased from 2% (Tilles et al. 1967) to up to 74% (Chonmaitree et al. 2000).

Often enteroviruses have been thought to cause mild diseases with characteristic signs (hand, foot, and mouth disease, herpangina) or more severe diseases such as meningitis. Although enteroviruses are known to cause upper respiratory infections, it was somewhat surprising that enteroviruses were associated with 25% of AOM events here (Study I). Because the diagnosis of enterovirus infections was previously made by very laborious viral culture, the search for enteroviruses was often excluded from studies of respiratory infections. Today, the RT-PCR method enables an easier detection of enteroviruses, promoting enteroviruses to be added to the detection panel of respiratory infections. A recent study reported that enteroviruses were found in 25% of children with URI, as detected by RT-PCR (Ruohola et al. 2000).

5.3.1 Concurrent virus detection in the nasopharynx and middle ear

In the FinOM Studies, the same virus was detected concurrently in the NPA sample and in at least one of the MEF specimens in 40% (Cohort Study) and 49% (Vaccine Trial) of AOM events. In both studies, RSV was most often detected concurrently in NPA and MEF samples, in up to 61% of RSV-positive AOM events. Rhinoviruses, enteroviruses, and influenza A viruses were present at similar frequency rates, in approximately 40% of AOM events, NPA and MEF were both positive.

As expected, in both FinOM Study groups, viral RNA or antigen was found more frequently in the NPA than in the MEF specimens. In almost one-half of the events with a NPA sample positive for a virus, the same virus was detected in the corresponding MEF sample. Interestingly, in 7% of AOM events, MEF was positive for a virus, while NPA remained negative.

There is little doubt that viruses have an important role in AOM, but it is not yet clear which factors are crucial for the development of AOM. Does the virus need to invade the middle ear and infect the middle ear mucosa, or would inflammation at the nasopharyngeal end of the tuba auditiva be sufficient to interfere with the innate defences of the middle ear mucosa and facilitate bacterial colonization? Viral infections have been shown to cause dysfunction of the Eustachian tube. Two-thirds of children develop abnormal middle ear pressure when they have a common cold (Winther et al. 2002). In adults, natural and experimental viral infection induce abnormal middle ear pressure and formation of middle ear effusion (Buchman et al. 1995; Arruda et al. 1997). In earlier studies, RSV has been suggested to be one of the most potent viruses contributing to the development of AOM (Henderson et al. 1982; Ruuskanen et al. 1989; Heikkinen et al. 1999). This appeared to be the case in the FinOM Studies. One could speculate that RSV causes more severe inflammatory responses, mucosal swelling, and dysfunction of the tuba auditiva. On the other hand, viral infection might facilitate colonization of the nasopharynx with pathogenic bacteria. Virus infection has been reported to increase the adherence of bacteria to pharyngeal cells (Hament et al. 1999).

In children, concurrent detection of viruses and bacteria in the MEF has been associated with prolonged clinical symptoms of AOM (Chonmaitree et al. 1990; Arola et al. 1990b; Chonmaitree et al. 1992). Despite adequate antibiotic drug compliance, viral

infection in children has been associated with an increased risk of AOM treatment failure (Patel et al. 1995). However, it is not known whether specific virus and bacteria combinations in MEF have certain impact on the development of AOM. In one study, *Streptococcus pneumoniae* was detected more often in the MEF containing influenza viruses than other viruses during AOM (Heikkinen et al. 1999). Combining results from several studies, more than half of AOM cases are positive for bacteria only, 5% for virus only, and 15% for both bacteria and virus; in 25% of cases, no pathogen was detected (Heikkinen et al. 2003a). Improved viral detection methods have, however, changed these proportions. According to the unpublished results of the FinOM Cohort Study, 32% of AOMs were positive for bacteria only, 19% for virus only, and 35% for both bacteria and virus. No middle ear pathogens were detected in 15% of cases. Ongoing development of detection methods will probably diminish the proportion of pathogen-negative AOMs since bacterial culture techniques are not sensitive enough for low bacterial concentrations (Virolainen et al. 1994), and all respiratory viruses are not included in the detection panels.

influenza virus type C, and human metapneumovirus, or an epidemic due to an unknown virus. In addition, the incidence of specific viruses can vary between years (Kaye et al. 1971; Waris 1991; Kim et al. 2000). For example, the proportion of AOM 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 only had a sensitivity of 66% as compared to direct RT-PCR used in the Vaccine Trial (Blomqvist et al. 1999).

5.3.2 Seasonal variation of viral findings in association with acute otitis media

Monthly rates of AOM events with coinciding viral infection showed similar seasonal variation in both FinOM Studies. The seasonal occurrence of virus-positive AOM events generally followed the incidence of AOM events. In both the FinOM Studies, the investigation of seasonal prevalence revealed a difference in winter 1996-97 between all AOM 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 such as coronavirus,

5.4 CHILDREN WITH FREQUENTLY RECURRING RESPIRATORY INFECTIONS (III)

In Study III, the 329 FinOM Cohort Study children were divided into subgroups according to the number of documented respiratory infections. Children who had experienced at least 9 URI episodes and/or at least 4 AOM episodes during the follow-up were designated children with FRRI; this group comprised 24% of children. The remaining children were considered non-FRRI children. As a group, the 78 FRRI children had 642 URI episodes (median rate 4.5 per person-year), and the 209 non-FRRI children with at least one URI episode had 716 URI episodes (median rate 1.7 per person-year). The median numbers of URI and AOM episodes

experienced in both groups of children are presented in Table 9.

To confirm that the very frequent referral of children to the study clinic was due to genuine viral infections, we also analyzed virus-positivity of URI episodes according to the number of URI episodes per child during the follow-up period (Figure 1). The overall virus-positivity was similar (median 56%) in all categories, suggesting that viral infections had an equal role in the etiology of upper respiratory infections irrespective of the frequency of infections.

Table 9. Demographic and clinical data of 329 Finnish Otitis Media Cohort Study children followed from 2 to 24 months of age.

	FRRI children ¹ n=78	Non-FRRI children n=251
Number of siblings:		
0	29 (37%)	133 (53%)
1	27 (35%)	79 (31%)
≥ 2 (range 2-6)	22 (28%)	39 (16%)
Type of day care:		
Home care	41 (53%)	166 (66%)
Family day care	6 (8%)	26 (10%)
Day care center	30 (38%)	52 (21%)
Other	1 (1%)	7 (3%)
≥ 2 siblings and/or day care	49 (63%)	78 (31%)
Median no. of URI episodes (range)	8 (4-13)	3 (0-8)
Median no. of AOM episodes (range)	4 (1-10)	0 (0-3)
Median age at time of first infection	145 days	214 days

¹ FRRI = frequently recurring respiratory infections, ≥9 upper respiratory infection (URI) episodes and/or ≥4 acute otitis media (AOM) episodes during the study period

As many as 63% of the FinOM Cohort Study children were cared for at home through out the study period, and by the end of the follow-up 25% of children attended a day-care center. Two or more siblings and/or attendance at day-care center were risk factors for recurrent infections (Table 9). These findings are in line with many reports that a large number of siblings and attendance at a day-care center increase the number of respiratory infections in children (Badger et al. 1953; Wald et al. 1988; Alho et al. 1990; Harsten et al. 1990; Tupasi et al. 1990; Louhiala et al. 1995; Celedon et al. 1999; Nafstad et al. 1999; Monto 2002). The infection-prone children could be speculated to have an innate susceptibility to recurrent infections, with exposure to other children providing a convenient environment for contracting different diseases. Genetic susceptibility has been suggested in a prospective twin and triplet cohort study of recurrent AOM (Casselbrant et al. 1999).

The FRR I group included more boys (58%) than the non-FRR I group (45%), but the difference was not statistically significant. It has been reported that boys are more prone to recurrent infections at least during the first years of life (Badger et al. 1953; Monto et al. 1974; Kim et al. 2000). No significant difference was present between FRR I and non-FRR I children with regard to proportions of premature birth, duration of breast-feeding, maternal smoking during pregnancy, parental household smoking, parental education, or single-parent households. However, it is notable that only a few FinOM Cohort Study families reported smoking indoors and that breast-feeding lasted at least 12 weeks in 71% of the families and at least 24 weeks in 51% of the families (Syrjänen et al. 2001).

Different definitions of FRR I can be found in the literature (Isaacs et al. 1982; Venuta et al. 1996; Chapelin et al. 1997; Giraudi et al. 1997; Hewson-Bower et al. 2001). In this Study III a pragmatic approach was used and single thresholds were selected in the rates of URI and

AOM episodes (Pitkäranta et al. 1999). The designated group of FRR I children appeared to have the expected characteristics, including a relatively higher number of siblings and attendance at day-care centers. As many as 48% of all of the documented URI episodes occurred in 78 children (24%) classified in the FRR I group. Analysis of the variation in the rate of URI revealed a continuum, without accurate segregation into “infection prone” or “normal” children suggesting, that the predetermined division into two groups may not be fully justified.

Structured investigation of recurrent respiratory infections is demanding, as the individual variation in the length and intensity of symptoms makes it difficult to determine whether a previous infection has ended before a new one commences. A standard 30-day length for AOM and URI episodes according to the study protocol was used (Vesa et al. 2001). The etiology of infections was analyzed first using clinical diagnosis and then virological findings as the starting points for the 30-day episodes. An additional approach in the analysis was based on dividing the children into an “infection-prone” quartile and control children according to the number of sick visits per study month. These three approaches yielded highly similar distributions of different viruses in the FRR I and non-FRR I groups (see below).

Although families were encouraged to take their child to the study clinic whenever the child was suffering from acute URI, the number of recorded URI episodes in this study was smaller than in some previous reports (Badger et al. 1953; Monto et al. 1974; Monto et al. 1993). The most important reason for this is that the study setting was very different from large community-based surveys, in which families were visited on a routine basis and even minor infections were recorded. Monto et al (1974) reported that in their study only every fourth respiratory illness resulted in a visit to a family physician. In the FinOM Cohort Study, 86% of

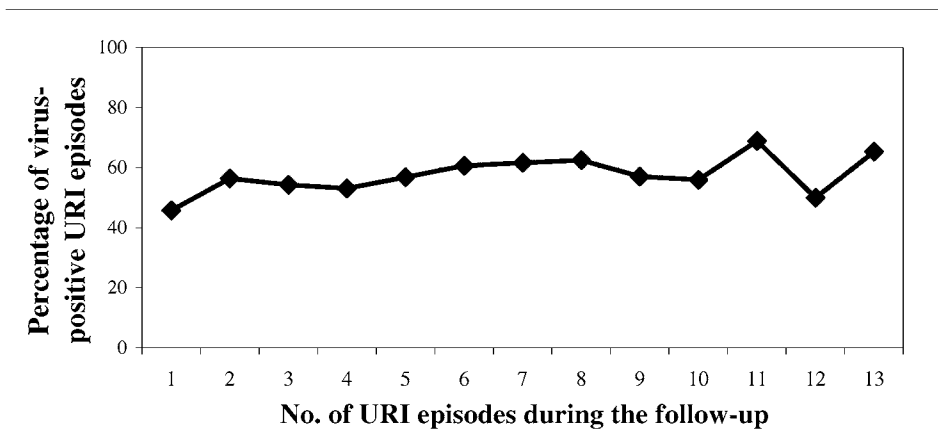
all AOM visits were captured at the study clinic (Kilpi et al. 2001). However, for 13% of children, no URI episodes were recorded at the study clinic, which seems to be relatively high percentage. Possibly children suffering from only mild symptoms were not always referred to the clinic. The inconvenience of a visit to the study clinic and fear of sample collection might also reduce the number of visits and recorded infections. Whether healthcare-seeking behavior for illness varied between the families is unknown. Working of the mothers was not analyzed, although it has been suggested that working mothers with child-care arranged outside the home are more likely than other mothers to seek medical care for minor symptoms (Horwitz et al. 1993). However, almost two-thirds of the study children were cared for at home, and since the overall virus-positivity of URIs did not vary significantly according to the number of reported URIs per child (Figure 1), this possible variation is unlikely to bias the results.

5.4.1 Viral etiology of frequently recurring respiratory infections

Designated URI episodes were used to assess possible sensitivity of FRRI children to specific virus infections. FRRI children had a slightly greater tendency to have rhinovirus-positive URI episodes than non-FRRI children (OR 1.58; 95% CI 1.27-1.96). The proportions of other viruses associated with URI episodes were similar in the two groups. Analysis of viral infections revealed that infection-prone children appear to pick up any viral infection present in the community.

Rhinoviruses were the most frequently diagnosed agents of URI among children with and without FRRI. However, rhinoviruses were detected by a combination of viral culture and RT-PCR, which may be a more sensitive method than the antigen detection technique used for the other viruses. Another bias is that serological diagnosis was attempted only at the time of AOM. However, only a minor proportion of virus diagnoses were made by serology, and in the FinOM Cohort Study, viral etiology of AOM was demonstrated to be similar to that of URI (Vesa et al. 2001).

Figure 1. Proportion of virus-positive upper respiratory infection (URI) episodes in children during the study follow-up period in the Finnish Otitis Media Cohort Study.



6 GENERAL DISCUSSION AND CONCLUSIONS

Viral respiratory infections are very common in children and are an enormous burden to the families and society. Present knowledge of the natural course and etiology of respiratory infections mainly comes from large community studies conducted several decades ago. Many studies of viral respiratory infections in children have since been carried out in different subsettings. In addition, advancements in diagnostic methods have made the detection of respiratory viruses more sensitive and convenient, which has in turn made the etiologic studies of respiratory infections easier. Although study settings and virus detection methods have varied between different studies, general characteristics and the main causative viral agents have remained similar over the years.

In the FinOM Studies, 940 children less than two years of age were analyzed for viral infections. These studies were prospective and very carefully planned and conducted. A unique material of over 7000 samples (NPA, MEF, and sera) for virological analysis enabled the study of many aspects of viral respiratory infections.

Several studies have been conducted to investigate the presence of viruses in AOM, but these two FinOM Studies together provide by far the largest study settings and sample collection on this matter. By using modern microbiological methods, including antigen detection and nucleic acid detection tests, a respiratory virus was found to be associated with two-thirds of AOM in children. Up to 41% of MEF specimens were positive for a virus. Picornaviruses were associated with over one-half of the AOM events. Although picornaviruses were detected by somewhat more sensitive method than other viruses, these findings are in agreement with other research, showing that rhinovirus infections in particular are very

common in young children. Interestingly, the proportion of specimens positive for enteroviruses was quite high. In a study using viral culture only, enteroviruses were one of the most common viruses detected from MEF during AOM (Chonmaitree et al. 1986). Enteroviruses have often been excluded from studies of respiratory infections since their detection was laborious and time-consuming. Recently, the RT-PCR method has made the detection of enteroviruses easier. Indeed, using this method, enteroviruses have been found in at least 25% of upper respiratory infections in children (Ruohola et al. 2000). In the FinOM Studies, RSV was associated with up to 10% of AOM events. This proportion would likely have been higher had PCR-based methods also been used for detection of RSV. RSV was most often detected concurrently in the nasopharynx and the MEF during AOM, suggesting that RSV might be more “ototropic” than other viruses, as has also been proposed by other researchers (Henderson et al. 1982; Heikkinen et al. 1999). In the future, the virus groups most commonly associated with AOM, i.e. picornaviruses and RSV, should be considered when aiming at prevention of AOM in children.

After primary virological analyses of the FinOM Cohort Study specimens, a large sample of NPA and MEF specimens was analyzed for human coronavirus RNA using the RT-PCR method. This method was adopted from earlier studies (Myint et al. 1994; Pitkäranta et al. 1997) and modified for detection of coronavirus RNA from large numbers of respiratory samples. One limitation in Study II was that the specimens in use were not randomly selected, and therefore, no statistical correlations between detected viruses and reported diagnoses were possible. Altogether, 2.4% of the NPA and MEF samples

were positive for coronavirus RNA. This is a low detection rate compared with another study in children using the RT-PCR method (Pitkäranta et al. 1998), although that study also noted that coronaviruses were uncommon in infants. Serological studies suggest that the frequency of human coronavirus infections in children less than 2 years of age may be relatively low (Hovi et al. 1979). Knowledge on coronavirus infections is still limited, and the data available are somewhat controversial. Most information is based on serological studies. During epidemics coronaviruses are claimed to be responsible for up to one-third of respiratory infections (Macnaughton et al. 1983), but seroepidemiologic studies have shown that asymptomatic infections are very common (Kaye et al. 1971). Moreover, coronavirus epidemics occur rarely, and during the FinOM Study period possibly no epidemics were present. In one relatively recent study, coronaviruses were associated with 4% of influenza-like illnesses in children less than 14 years of age, as detected by the antigen test (Lina et al. 1996). Human coronavirus infections are probably not very common in young children, whereas later in life, clinical and subclinical infections occur more often.

Physicians are very familiar with the children with frequent respiratory infections phenomenon. These children seem to catch every respiratory virus present in the community, and especially during the winter, to be sick “all the time”. These children are otherwise healthy, recover from infections normally, and their growth and development is normal. Previous reports have suggested that infection-prone children have reduced production of IFN- α , which is an important part of the immune defence mechanisms against viral infections (Isaacs et al. 1981; Pitkäranta et al. 1993). The question therefore arises of whether children with recurrent respiratory infections might be more susceptible to infections caused by specific

viruses. Analysis of upper respiratory infections in the FinOM Cohort Study revealed that the distribution of specific virus infections was similar among children with and without FRRI. Only the proportion of rhinovirus infections was slightly higher among infection-prone children. As expected, the causes for recurrence of infections are diverse. One might speculate that due to unknown, and maybe mild, deficiencies in immune defence some children have a host-derived susceptibility to viral infections. Environmental factors, such as exposure to other children or tobacco smoke, may also increase the infection pressure of attacking viruses or weaken the defence barriers. Research on this topic has helped families and communities to take actions to reduce the effect of environmental factors; these include increasing hand washing, avoiding smoking inside the house, and limiting the number of children in day-care groups. However, no methods for improving the host-derived ability to endure the respiratory viruses with which young children are bombarded exist to date.

As the use of the very sensitive PCR-based detection method has increased, the question has been posed as to whether PCR methods are too sensitive, detecting small remnants of viruses that probably have no clinical relevance. This question drove us to investigate the presence of viral RNA in the nasopharynx of children without respiratory symptoms. In Study IV, as many as 29% of the children were positive for viral RNA. However, most of the findings were linked to previous or future respiratory symptoms, and only 4 of the 31 children with virus-positive samples had no recent connection to respiratory infection. An interesting question then is why was viral RNA detected in these children? Is the virus a long-lasting but not active remnant of a past infection or is there a “silent” infection in which the virus replicates at a low level. Or is it a subclinical infection in which the viral load and infectious

activity are high, but the individual manifests no clinical symptoms? It has been reported that clinical symptoms are largely cytokine-mediated, and therefore, symptomatic disease may continue even after viral load diminishes (Hendley 1999). The introduction of PCR methods into clinical practice has resulted in the same questions and problems of clinical relevance as for other diagnostic methods. Cooney et al. showed that the amount of viral culture is highest at the onset of respiratory infection (Cooney et al. 1972). In clinical practice, laboratory findings must always be interpreted in association with clinical signs and symptoms. Therefore, detection of a respiratory viral nucleic acid by (RT-) PCR in the nasopharynx must also be interpreted cautiously because the presence of a virus alone does not establish causality of the concurrent illness. However, Study IV did demonstrate that in most cases viral RNA detection can be linked to past or future respiratory symptoms. Thus, the vast majority of viruses detected in patients can be assumed to be involved in the initiation of the observed respiratory infection.

Conclusions

1. In two large prospective cohort studies (FinOM Studies), respiratory viruses were shown to be present in two-thirds of AOM events; in half of these, a picornavirus infection was detected.
2. Human coronavirus RNA was detected by RT-PCR from 2.4% of NPA and/or MEF specimens of children less than 2 years of age.
3. A specific susceptibility to a defined type or group of viruses does not seem to be a major explanatory factor for frequently recurring respiratory infections in young children. Exposure to other children (siblings and/or attendance to day care) was associated with recurrence of infections.
4. In asymptomatic children, detection of picornavirus RNA by the sensitive RT-PCR method is relatively common in the context of past (or future) respiratory infection but is not common in children without recent exposure to respiratory symptoms.

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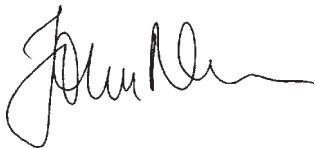
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REFERENCES

- Alho, O., M. Koivu, M. Sorri and P. Rantakallio. Risk factors for recurrent acute otitis media and respiratory infection in infancy. *Int J Pediatr Otorhinolaryngol* 19: 151-161, 1990
- Andreoletti, L., M. Lesay, A. Deschildre, V. Lambert, A. Dewilde and P. Wattre. Differential detection of rhinoviruses and enteroviruses RNA sequences associated with classical immunofluorescence assay detection of respiratory virus antigens in nasopharyngeal swabs from infants with bronchiolitis. *J Med Virol* 61(3): 341-6, 2000
- Arifeen, S., R. E. Black, G. Antelman, A. Baqui, L. Caulfield and S. Becker. Exclusive breastfeeding reduces acute respiratory infection and diarrhea deaths among infants in Dhaka slums. *Pediatrics* 108(4): e67, 2001
- Arola, M., O. Ruuskanen, T. Ziegler, J. Mertsola, K. Nanto-Salonen, A. Putto-Laurila, M. K. Viljanen and P. Halonen. Clinical role of respiratory virus infection in acute otitis media. *Pediatrics* 86(6): 848-55, 1990a
- Arola, M., T. Ziegler and O. Ruuskanen. Respiratory virus infection as a cause of prolonged symptoms in acute otitis media. *J Pediatr* 116(5): 697-701, 1990b
- Arruda, d. E., F. G. Hayden, J. F. McAuliffe, M. A. de Sousa, S. B. Mota, M. I. McAuliffe, F. C. Geist, E. P. Carvalho, M. C. Fernandes, R. L. Guerrant and et al. Acute respiratory viral infections in ambulatory children of urban northeast Brazil. *J Infect Dis* 164(2): 252-8, 1991
- Arruda, E., A. Pitkäranta, T. J. J. Wittek, C. A. Doyle and F. G. Hayden. Frequency and natural history of rhinovirus infections in adults during Autumn. *J Clin Microbiol* 35(11): 2864-8, 1997
- Avellon, A., P. Perez, J. C. Aguilar, R. Lejarazu and J. E. Echevarria. Rapid and sensitive diagnosis of human adenovirus infections by a generic polymerase chain reaction. *J Virol Methods* 92(2): 113-20, 2001
- Badger, G., J. Dingle, A. Feller, R. Hodges, W. j. Jordan and C. j. Rammelkamp. A study of illness in a group of Cleveland families. II. Incidence of the common respiratory diseases. *Am J Hyg* 58: 31-40, 1953
- Beard, L. J., G. M. Maxwell and Y. H. Thong. Immunocompetence of children with frequent respiratory infections. *Arch Dis Child* 56(2): 101-5, 1981
- Berglund, B., A. Salmivalli and P. Toivanen. Isolation of respiratory syncytial virus from middle ear exudates of infants. *Acta Otolaryngol* 61(6): 475-487, 1966
- Bitnun, A., U. Allen, H. Heurter, S. M. King, M. A. Opavsky, E. L. Ford-Jones, A. Matlow, I. Kitai, R. Tellier, S. Richardson, D. Manson, P. Babyn and S. Read. Children hospitalized with severe acute respiratory syndrome-related illness in Toronto. *Pediatrics* 112(4): e261, 2003
- Blomqvist, S., M. Roivainen, T. Puhakka, M. Kleemola and T. Hovi. Virological and serological analysis of rhinovirus infections during the first two years of life in a cohort of children. *J Med Virol* 66(2): 263-8., 2002a
- Blomqvist, S., C. Savolainen, L. Raman, M. Roivainen and T. Hovi. Human rhinovirus 87 and enterovirus 68 represent a unique serotype with rhinovirus and enterovirus features. *J Clin Microbiol* 40(11): 4218-23, 2002b
- Blomqvist, S., A. Skyttä, M. Roivainen and T. Hovi. Rapid detection of human rhinovirus in nasopharyngeal aspirates by a microwell reverse transcription-PCR-hybridization assay. *J Clin Microbiol* 37(9): 2813-2816, 1999

-
- Boivin, G., G. De Serres, S. Cote, R. Gilca, Y. Abed, L. Rochette, M. G. Bergeron and P. Dery. Human metapneumovirus infections in hospitalized children. *Emerg Infect Dis* 9(6): 634-40, 2003
- Boivin, G., A. D. Osterhaus, A. Gaudreau, H. C. Jackson, J. Groen and P. Ward. Role of picornaviruses in flu-like illnesses of adults enrolled in an oseltamivir treatment study who had no evidence of influenza virus infection. *J Clin Microbiol* 40(2): 330-4, 2002
- Bramley, T. J., D. Lerner and M. Sames. Productivity losses related to the common cold. *J Occup Environ Med* 44(9): 822-9, 2002
- Bryd, R. S. and C. R. Howard. Children's passive and prenatal exposure to cigarette smoke. *Pediatr Ann* 24: 640-645, 1995
- Buchman, C. A., W. J. Doyle, D. P. Skoner, J. C. Post, C. M. Alper, J. T. Seroky, K. Anderson, R. A. Preston, F. G. Hayden, P. Fireman and et al. Influenza A virus-induced acute otitis media. *J Infect Dis* 172(5): 1348-51, 1995
- Casey, H., U. Kokko, J. Schubert and H. Woodworth. Standardized diagnostic complement fixation method and adaptation to micro test. Washington, DC, Department of Health, Education and Welfare, 1965
- Casselbrant, M. L., E. M. Mandel, P. A. Fall, H. E. Rockette, M. Kurs-Lasky, C. D. Bluestone and R. E. Ferrell. The heritability of otitis media. A twin and triplet study. *JAMA* 282(22): 2125-2130, 1999
- Celedon, J. C., A. A. Litonjua, S. T. Weiss and D. R. Gold. Day care attendance in the first year of life and illnesses of the upper and lower respiratory tract in children with a familial history of atopy. *Pediatrics* 104(3 Pt 1): 495-500, 1999
- Chan, L. G., U. D. Parashar, M. S. Lye, F. G. Ong, S. R. Zaki, J. P. Alexander, K. K. Ho, L. L. Han, M. A. Pallansch, A. B. Suleiman, M. Jegathesan and L. J. Anderson. Deaths of children during an outbreak of hand, foot, and mouth disease in Sarawak, Malaysia: clinical and pathological characteristics of the disease. *Clin Infect Dis* 31(3): 678-83, 2000
- Chapelin, C., A. Coste, P. Reinert, A. Boucherat, M.-L. Millepied, F. Poron and E. Escudier. Incidence of primary ciliary dyskinesia in children with recurrent respiratory diseases. *Ann Otol Rhinol Laryngol* 106: 854-858, 1997
- Chidekel, A. S., C. L. Rosen and A. R. Bazzzy. Rhinovirus infection associated with serious lower respiratory illness in patients with bronchopulmonary dysplasia. *Pediatr Infect Dis J* 16(1): 43-7, 1997
- Chonmaitree, T. and K. J. Henrickson. Detection of respiratory viruses in the middle ear fluids of children with acute otitis media by multiplex reverse transcription: polymerase chain reaction assay. *Pediatr Infect Dis J* 19(3): 258-60, 2000
- Chonmaitree, T., V. M. Howie and A. L. Truant. Presence of respiratory viruses in middle ear fluids and nasal wash specimens from children with acute otitis media. *Pediatrics* 77(5): 698-702., 1986
- Chonmaitree, T., M. J. Owen and V. M. Howie. Respiratory viruses interfere with bacteriologic response to antibiotic in children with acute otitis media. *J Infect Dis* 162(2): 546-9, 1990
- Chonmaitree, T., M. J. Owen, J. A. Patel, D. Hedgpeth, D. Horlick and V. M. Howie. Effect of viral respiratory tract infection on outcome of acute otitis media. *J Pediatr* 120(6): 856-62, 1992
- Christopher, S., S. Theogaraj, A. Godbole and J. John. An epidemic of acute hemorrhagic conjunctivitis due to coxsackievirus A24. *J Infect Dis* 146(1): 16-19, 1982

-
- Cooney, M. K., C. E. Hall and J. P. Fox. The Seattle virus watch. 3. Evaluation of isolation methods and summary of infections detected by virus isolations. *Am J Epidemiol* 96(4): 286-305, 1972
- Covalciuc, K. A., K. H. Webb and C. A. Carlson. Comparison of four clinical specimen types for detection of influenza A and B viruses by optical immunoassay (FLU OIA test) and cell culture methods. *J Clin Microbiol* 37(12): 3971-4, 1999
- Cyranoski, D. Health labs focus on mystery pneumonia. *Nature* 422(6929): 247, 2003
- Denison, M. R. The common cold: rhinoviruses and coronaviruses. *Viral infections of the respiratory tract*. P. F. Wright, Ed. New York, Marcel Dekker Inc. 127: 253-280, 1999
- Douglas, R. M., H. Miles, D. Hansman, A. Fadejevs, B. Moore and M. D. Bollen. Acute tonsillitis in children: microbial pathogens in relation to age. *Pathology* 16(1): 79-82, 1984
- Drosten, C., S. Gunther, W. Preisner, S. van der Werf, H. R. Brodt, S. Becker, H. Rabenau, M. Panning, L. Kolesnikova, R. A. Fouchier, A. Berger, A. M. Burguiere, J. Cinatl, M. Eickmann, N. Escriou, K. Grywna, S. Kramme, J. C. Manuguerra, S. Muller, V. Rickerts, M. Sturmer, S. Vieth, H. D. Klenk, A. D. Osterhaus, H. Schmitz and H. W. Doerr. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med* 348(20): 1967-76, 2003
- Drummond, P. D. and B. Hewson-Bower. Increased psychosocial stress and decreased mucosal immunity in children with recurrent upper respiratory tract infections. *J Psychosom Res* 43(3): 271-8, 1997
- Ebihara, T., R. Endo, H. Kikuta, N. Ishiguro, M. Yoshioka, X. Ma and K. Kobayashi. Seroprevalence of human metapneumovirus in Japan. *J Med Virol* 70(2): 281-3, 2003
- Eskola, J., T. Kilpi, A. Palmu, J. Jokinen, J. Haapakoski, E. Herva, A. Takala, H. Käyhty, P. Karma, H. Mäkelä, R. Kohberger and G. Siber. Efficacy of a sevenvalent pneumococcal polysaccharide-CRM197 conjugate vaccine against serotype-specific, culture-confirmed pneumococcal acute otitis media in infants and children. *New Engl J Med* 344(6): 403-409, 2001
- Esper, F., R. A. Martinello, D. Boucher, C. Weibel, D. Ferguson, M. L. Landry and J. S. Kahn. A 1-year experience with human metapneumovirus in children aged <5 years. *J Infect Dis* 189(8): 1388-96, 2004
- Faden, H., L. Duffy, R. Wasielewski, J. Wolf, D. Krystofik and Y. Tung. Relationship between nasopharyngeal colonization and the development of otitis media in children. *J Infect Dis* 175: 1440-5, 1997
- Freytmuth, F., J. Petitjean, P. Pothier, J. Brouard and E. Norrby. Prevalence of respiratory syncytial virus subgroups A and B in France from 1982 to 1990. *J Clin Microbiol* 29(3): 653-5, 1991
- Frost, W. H. and M. Gover. The incidence and time distribution of common colds in several groups kept under continuous observation. *Pub Health Rep* 47: 1815-41, 1932
- Gern, J. E. and W. W. Busse. The role of viral infections in the natural history of asthma. *J Allergy Clin Immunol* 106(2): 201-12, 2000
- Ghazi, F., P. J. Hughes, T. Hyypiä and G. Stanway. Molecular analysis of human parechovirus type 2 (formerly echovirus 23). *J Gen Virol* 79 (Pt 11): 2641-50, 1998
- Gill, E. P., E. A. Dominguez, S. B. Greenberg, R. L. Atmar, B. G. Hogue, B. D. Baxter and R. B. Couch. Development and application of an enzyme immunoassay for coronavirus OC43 antibody in acute respiratory illness. *J Clin Microbiol* 32(10): 2372-6, 1994

-
- Giraudi, V., C. Riganti, M. R. Torales, H. Sedola and E. Gaddi. Upper respiratory infections in children: response to endonasal administration of IGA. *Int J Pediatr Otorhinolaryngol* 39(2): 103-10, 1997
- Goldmann, D. A. Transmission of viral respiratory infections in the home. *Pediatr Infect Dis J* 19(10 Suppl): S97-102, 2000
- Gonzales, R., D. C. Malone, J. H. Maselli and M. A. Sande. Excessive antibiotic use for acute respiratory infections in the United States. *Clin Infect Dis* 33(6): 757-62, 2001
- Gröndahl, B., W. Puppe, A. Hoppe, I. Kuhne, J. A. Weigl and H. J. Schmitt. Rapid identification of nine microorganisms causing acute respiratory tract infections by single-tube multiplex reverse transcription-PCR: feasibility study. *J Clin Microbiol* 37(1): 1-7, 1999
- Grönroos, J. A., A. E. Kortekangas, L. Ojala and M. Vuori. The aetiology of acute middle ear infection. *Acta Otolaryngol* 58: 149-158, 1964
- Grönroos, J. A., L. Vihma, A. Salmivalli and B. Berglund. Coexisting viral (respiratory syncytial) and bacterial (Pneumococcus) otitis media in children. *Acta Otolaryngol* 65(505-517), 1968
- Guill, M. Respiratory manifestations of gastroesophageal reflux in children. *J Asthma* 32(3): 173-189, 1995
- Gwaltney, J. M. Clinical significance and pathogenesis of viral respiratory infections. *Am J Med* 112 Suppl 6A: 13S-18S, 2002
- Hall, C. B. Respiratory syncytial virus and parainfluenza virus. *N Engl J Med* 344(25): 1917-28, 2001a
- Hall, C. B., C. E. Long and K. C. Schnabel. Respiratory syncytial virus infections in previously healthy working adults. *Clin Infect Dis* 33(6): 792-6, 2001b
- Halonen, P., O. Meurman, T. Lövgren, I. Hemmälä and E. Soini. Detection of viral antigens by time-resolved fluoroimmunoassay. *Curr Topics Microbiol Immunol* 104: 133-146, 1983
- Hament, J. M., J. L. Kimpen, A. Fleer and T. F. Wolfs. Respiratory viral infection predisposing for bacterial disease: a concise review. *FEMS Immunol Med Microbiol* 26(3-4): 189-95, 1999
- Hamory, B. H., M. A. Sande, A. Sydnor, Jr., D. L. Seale and J. M. Gwaltney, Jr. Etiology and antimicrobial therapy of acute maxillary sinusitis. *J Infect Dis* 139(2): 197-202, 1979
- Harsten, G., K. Prellner, J. Heldrup, O. Kalm and R. Kornfält. Acute respiratory tract infections in children. *Acta Paediatr Scand* 70: 402-409, 1990
- Hayden, F. G. Influenza virus and rhinovirus-related otitis media: potential for antiviral intervention. *Vaccine* 19: S66-S70, 2001
- Hayden, F. G. Rhinovirus and the lower respiratory tract. *Rev Med Virol* 14(1): 17-31, 2004
- Hayden, F. G., J. K. Albrecht, D. L. Kaiser and J. M. Gwaltney, Jr. Prevention of natural colds by contact prophylaxis with intranasal alpha 2-interferon. *N Engl J Med* 314(2): 71-5, 1986
- Hayden, F. G., D. T. Herrington, T. L. Coats, K. Kim, E. C. Cooper, S. A. Villano, S. Liu, S. Hudson, D. C. Pevear, M. Collett and M. McKinlay. Efficacy and safety of oral pleconaril for treatment of colds due to picornaviruses in adults: results of 2 double-blind, randomized, placebo-controlled trials. *Clin Infect Dis* 36(12): 1523-32, 2003
- Hazlett, D. T., T. M. Bell, P. M. Tukei, G. R. Adamba, W. O. Ochieng, J. M. Magana, G. W. Gathara, E. M. Wafula, A. Pamba, J. O. Ndinya-Achola and et al. Viral etiology and epidemiology of acute respiratory infections in children in Nairobi, Kenya. *Am J Trop Med Hyg* 39(6): 632-40, 1988

-
- Heikkinen, T. and T. Chonmaitree. Importance of respiratory viruses in acute otitis media. *Clin Microbiol Rev* 16(2): 230-41, 2003a
- Heikkinen, T. and A. Järvinen. The common cold. *Lancet* 361(9351): 51-9, 2003b
- Heikkinen, T., J. Marttila, A. A. Salmi and O. Ruuskanen. Nasal swab versus nasopharyngeal aspirate for isolation of respiratory viruses. *J Clin Microbiol* 40(11): 4337-9, 2002
- Heikkinen, T., O. Ruuskanen, M. Waris, T. Ziegler, M. Arola and P. Halonen. Influenza vaccination in the prevention of acute otitis media in children. *Am J Dis Child* 145: 445-8, 1991
- Heikkinen, T., M. Thint and T. Chonmaitree. Prevalence of various respiratory viruses in the middle ear during acute otitis media. *N Engl J Med* 340(4): 260-4, 1999
- Heikkinen, T., M. Waris, O. Ruuskanen, A. Putto-Laurila and J. Mertsola. Incidence of acute otitis media associated with group A and B respiratory syncytial virus infections. *Acta Paediatr* 84(4): 419-23, 1995
- Henderson, F. W., A. M. Collier, M. A. Sanyal, J. M. Watkins, D. L. Fairclough, W. A. Clyde, Jr. and F. W. Denny. A longitudinal study of respiratory viruses and bacteria in the etiology of acute otitis media with effusion. *N Engl J Med* 306(23): 1377-83., 1982
- Hendley, J. O. Clinical virology of rhinoviruses. *Adv Virus Res* 54: 453-66, 1999
- Hendley, J. O. and J. M. Gwaltney, Jr. Mechanisms of transmission of rhinovirus infections. *Epidemiol Rev* 10: 243-58, 1988
- Henkel, J. H., S. W. Aberle, M. Kundi and T. Popow-Kraupp. Improved detection of respiratory syncytial virus in nasal aspirates by seminested RT-PCR. *J Med Virol* 53(4): 366-71, 1997
- Hewson-Bower, B. and P. D. Drummond. Psychological treatment for recurrent symptoms of colds and flu in children. *J Psychosom Res* 51(1): 369-77, 2001
- Hierholzer, J. C., P. G. Bingham, R. A. Coombs, K. H. Johansson, L. J. Anderson and P. E. Halonen. Comparison of monoclonal antibody time-resolved fluoroimmunoassay with monoclonal antibody capture-biotinylated detector enzyme immunoassay for respiratory syncytial virus and parainfluenza virus antigen detection. *J Clin Microbiol* 27(6): 1243-9, 1989
- Hierholzer, J. C., P. Halonen, P. G. Bingham, R. A. Coombs and Y. O. Stone. Antigen detection in human respiratory Coronavirus infections by monoclonal time-resolved fluoroimmunoassay. *Clin Diagn Virol* 2: 165-179, 1994
- Hierholzer, J. C., K. H. Johansson, L. J. Anderson, C. J. Tsou and P. E. Halonen. Comparison of monoclonal time-resolved fluoroimmunoassay with monoclonal capture-biotinylated detector enzyme immunoassay for adenovirus antigen detection. *J Clin Microbiol* 25(9): 1662-7., 1987
- Hirsilä, M., J. Kauppila, K. Tuomaala, B. Grekula, T. Puhakka, O. Ruuskanen and T. Ziegler. Detection by reverse transcription-polymerase chain reaction of influenza C in nasopharyngeal secretions of adults with a common cold. *J Infect Dis* 183(8): 1269-72., 2001
- Hon, K. L., C. W. Leung, W. T. Cheng, P. K. Chan, W. C. Chu, Y. W. Kwan, A. M. Li, N. C. Fong, P. C. Ng, M. C. Chiu, C. K. Li, J. S. Tam and T. F. Fok. Clinical presentations and outcome of severe acute respiratory syndrome in children. *Lancet* 361(9370): 1701-3, 2003
- Hong, J. Y., H. J. Lee, P. A. Piedra, E. H. Choi, K. H. Park, Y. Y. Koh and W. S. Kim. Lower respiratory tract infections due to adenovirus in hospitalized Korean children: epidemiology, clinical features, and prognosis. *Clin Infect Dis* 32(10): 1423-9, 2001

-
- van den Hoogen, B. G., J. C. de Jong, J. Groen, T. Kuiken, R. de Groot, R. A. Fouchier and A. D. Osterhaus. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 7(6): 719-24., 2001
- Horn, M., E. Brain, I. Gregg, J. Inglis, S. Yealland and P. Taylor. Respiratory viral infection and wheezy bronchitis in childhood. *Thorax* 34: 23-28, 1979
- Horwitz, S. M., R. I. Horwitz and H. Morgenstern. Maternal employment, maternal care and pediatric visits for minor acute illnesses. *J Clin Epidemiol* 46(9): 981-6, 1993
- Howe, M. Australian find suggests worldwide reach for metapneumovirus. *Lancet Infect Dis* 2(4): 202, 2002
- Hovi, T., H. Kainulainen, B. Ziola and A. Salmi. OC43 strain-related coronavirus antibodies in different age groups. *J Med Virol* 3(4): 313-20, 1979
- Hovi, T., M. Stenvik and M. Rosenlew. Relative abundance of enterovirus serotypes in sewage differs from that in patients: clinical and epidemiological implications. *Epidemiol Infect* 116(1): 91-7, 1996
- Hyypiä, T., C. Horsnell, M. Maaronen, M. Khan, N. Kalkkinen, P. Auvinen, L. Kinnunen and G. Stanway. A distinct picornavirus group identified by sequence analysis. *Proc Natl Acad Sci USA* 89(18): 8847-51, 1992
- Hyypiä, T., T. Puhakka, O. Ruuskanen, M. Mäkelä, A. Arola and P. Arstila. Molecular diagnosis of human rhinovirus infections: comparison with virus isolation. *J Clin Microbiol* 36(7): 2081-3, 1998
- Ieven, M. and H. Goossens. Relevance of nucleic acid amplification techniques for diagnosis of respiratory tract infections in the clinical laboratory. *Clin Microbiol Rev* 10(2): 242-256, 1997
- The IMPact-RSV Study Group. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. The IMPact-RSV Study Group. *Pediatrics* 102(3 Pt 1): 531-7, 1998
- Isaacs, D., J. R. Clarke, D. A. J. Tyrrell and H. B. Valman. Selective infection of lower respiratory tract by respiratory viruses in children with recurrent respiratory tract infections. *BMJ* 284(12): 1746-1748, 1982
- Isaacs, D., J. R. Clarke, D. A. J. Tyrrell, A. D. B. Webster and H. B. Valman. Deficient production of leucocyte interferon (interferon-alfa) in vitro and in vivo in children with recurrent respiratory tract infections. *Lancet* 31: 950-952, 1981
- Isaacs, D., D. Flowers, J. R. Clarke, H. B. Valman and M. R. MacNaughton. Epidemiology of coronavirus respiratory infections. *Arch Dis Child* 58(7): 500-3., 1983
- Isaia, G., O. Teodosiu, G. Popescu, P. Athanasiu, I. Sternberg and Z. Dumitriu. Persistence of viruses in the nasopharynx of apparently healthy children aged 0-5 years. Results of investigations performed in 1982-83. *Virology* 36(3): 175-9, 1985
- Ison, M., J. Mills, P. Openshaw, M. Zambon, A. Osterhaus and F. Hayden. Current research on respiratory viral infections: Fourth International Symposium. *Antiviral Res* 55(2): 227-78, 2002
- Jaakkola, J. J., M. Paunio, M. Virtanen and O. P. Heinonen. Low-level air pollution and upper respiratory infections in children. *Am J Public Health* 81(8): 1060-1063, 1991
- Jain, A., A. Pande, P. K. Misra, A. Mathur and U. C. Chaturvedi. An Indian hospital study of viral causes of acute respiratory infection in children. *J Med Microbiol* 35(4): 219-23, 1991

-
- Jartti, T., B. van den Hoogen, R. P. Garofalo, A. D. Osterhaus and O. Ruuskanen. Metapneumovirus and acute wheezing in children. *Lancet* 360(9343): 1393-4, 2002
- Jennings, L., G. Barns and K. Dawson. The association of viruses with acute asthma. *New Zeal Med J* 12: 488-490, 1987
- Johnston, S., G. Sanderson, P. Pattemore, S. Smith, P. Bardin, C. Bruce, P. Lambden, D. Tyrrell and S. Holgate. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. *J Clin Microbiol* 31(1): 111-117, 1993
- Johnston, S. L., P. K. Pattemore, G. Sanderson, S. Smith, F. Lampe, L. Josephs, P. Symington, S. O'Toole, S. H. Myint, D. A. Tyrrell and et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *BMJ* 310(6989): 1225-9, 1995
- Joki-Erkkilä, V. P., P. Laippala and J. Pukander. Increase in paediatric acute otitis media diagnosed by primary care in two Finnish municipalities -1994-5 versus 1978-9. *Epidemiol Infect* 121(3): 529-34, 1998
- Joki-Korpela, P. and T. Hyypiä. Diagnosis and epidemiology of echovirus 22 infections. *Clin Infect Dis* 27(1): 129-36, 1998
- Joki-Korpela, P. and T. Hyypiä. Parechoviruses, a novel group of human picornaviruses. *Ann Med* 33(7): 466-71., 2001
- Juvén, T., J. Mertsola, M. Waris, M. Leinonen, O. Meurman, M. Roivainen, J. Eskola, P. Saikku and O. Ruuskanen. Etiology of community-acquired pneumonia in 254 hospitalized children. *Pediatr Infect Dis J* 19(4): 293-8, 2000
- Karma, P., T. Palva, K. Kouvalainen, J. Karja, P. Mäkelä, V. Prinssi, O. Ruuskanen and K. Launiala. Finnish approach to the treatment of acute otitis media. Report of the Finnish Consensus Conference. *Ann Otol Rhinol Laryngol Suppl* 129: 1-19, 1987
- Karron, R. A., R. B. Belshe, P. F. Wright, B. Thumar, B. Burns, F. Newman, J. C. Cannon, J. Thompson, T. Tsai, M. Paschalis, S. L. Wu, Y. Mitcho, J. Hackell, B. R. Murphy and J. M. Tatem. A live human parainfluenza type 3 virus vaccine is attenuated and immunogenic in young infants. *Pediatr Infect Dis J* 22(5): 394-405, 2003
- Kawasaki, Y., M. Hosoya, M. Katayose and H. Suzuki. Correlation between serum interleukin 6 and C-reactive protein concentrations in patients with adenoviral respiratory infection. *Pediatr Infect Dis J* 21(5): 370-4, 2002
- Kaye, H. S., H. B. Marsh and W. R. Dowdle. Seroepidemiologic survey of coronavirus (strain OC 43) related infections in a children's population. *Am J Epidemiol* 94(1): 43-9, 1971
- Kilpi, T., H. Ahman, J. Jokinen, K. S. Lankinen, A. Palmu, H. Savolainen, M. Gronholm, M. Leinonen, T. Hovi, J. Eskola, H. Kayhty, N. Bohidar, J. C. Sadoff and P. H. Makela. Protective efficacy of a second pneumococcal conjugate vaccine against pneumococcal acute otitis media in infants and children: randomized, controlled trial of a 7-valent pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine in 1666 children. *Clin Infect Dis* 37(9): 1155-64, 2003
- Kilpi, T., E. Herva, T. Kaijalainen, R. Syrjanen and A. K. Takala. Bacteriology of acute otitis media in a cohort of Finnish children followed for the first two years of life. *Pediatr Infect Dis J* 20(7): 654-62., 2001
- Kim, J. O. and R. L. Hodinka. Serious respiratory illness associated with rhinovirus infection in a pediatric population. *Clin Diagn Virol* 10(1): 57-65, 1998

-
- Kim, M. R., H. R. Lee and G. M. Lee. Epidemiology of acute viral respiratory tract infections in Korean children. *J Infect* 41(2): 152-8, 2000
- King, A. M. Q., F. Brown, P. Christian, T. Hovi, T. Hyypia, N. J. Knowles, S. M. Lemon, P. D. Minor, A. C. Palmenberg, T. Skern and G. Stanway. Picornaviridae. *Virus Taxonomy*. M. H. V. van Regenmortel, C. M. Fauquet, D. H. L. Bishop et al, Eds. San Diego, Academic Press: 657-678, 2000
- Klein, B. S., F. R. Dollete and R. H. Yolken. The role of respiratory syncytial virus and other viral pathogens in acute otitis media. *J Pediatr* 101(1): 16-20, 1982
- Knott, A. M., C. E. Long and C. B. Hall. Parainfluenza viral infections in pediatric outpatients: seasonal patterns and clinical characteristics. *Pediatr Infect Dis J* 13(4): 269-73, 1994
- Koch, A., M. Melbye, P. Sorensen, P. Homoe, H. O. Madsen, K. Molbak, C. H. Hansen, L. H. Andersen, G. W. Hahn and P. Garred. Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. *JAMA* 285(10): 1316-21., 2001
- Kramer, M. S., B. Chalmers, E. D. Hodnett, Z. Sevkovskaya, I. Dzikovich, S. Shapiro, J. P. Collet, I. Vanilovich, I. Mezen, T. Ducruet, G. Shishko, V. Zubovich, D. Mknuk, E. Gluchanina, V. Dombrovskiy, A. Ustinovitch, T. Kot, N. Bogdanovich, L. Ovchinikova and E. Helsing. Promotion of Breastfeeding Intervention Trial (PROBIT): a randomized trial in the Republic of Belarus. *JAMA* 285(4): 413-20., 2001
- Kristo, A., M. Uhari, J. Luotonen, P. Koivunen, E. Ilkko, T. Tapiainen and O. P. Alho. Paranasal sinus findings in children during respiratory infection evaluated with magnetic resonance imaging. *Pediatrics* 111(5 Pt 1): e586-9, 2003
- Ksiazek, T. G., D. Erdman, C. S. Goldsmith, S. R. Zaki, T. Peret, S. Emery, S. Tong, C. Urbani, J. A. Comer, W. Lim, P. E. Rollin, S. F. Dowell, A. E. Ling, C. D. Humphrey, W. J. Shieh, J. Guarner, C. D. Paddock, P. Rota, B. Fields, J. DeRisi, J. Y. Yang, N. Cox, J. M. Hughes, J. W. LeDuc, W. J. Bellini and L. J. Anderson. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* 348(20): 1953-66, 2003
- Lina, B., M. Valette, S. Foray, J. Luciani, J. Stagnara, D. M. See and M. Aymard. Surveillance of community-acquired viral infections due to respiratory viruses in Rhone-Alpes (France) during winter 1994 to 1995. *J Clin Microbiol* 34(12): 3007-11, 1996
- López-Alarcón, M., S. Villalpando and A. Fajardo. Breast-feeding lowers the frequency and duration of acute respiratory infection and diarrhea in infants under six months of age. *J Nutr* 127(3): 436-43., 1997
- Louhiala, P. J., N. Jaakkola, R. Ruotsalainen and J. J. Jaakkola. Form of day care and respiratory infections among Finnish children. *Am J Public Health* 85(8 Pt 1): 1109-12, 1995
- Lu, C. Y., C. Y. Lee, C. L. Kao, W. Y. Shao, P. I. Lee, S. J. Twu, C. C. Yeh, S. C. Lin, W. Y. Shih, S. I. Wu and L. M. Huang. Incidence and case-fatality rates resulting from the 1998 enterovirus 71 outbreak in Taiwan. *J Med Virol* 67(2): 217-23, 2002
- Macnaughton, M. R. Occurrence and frequency of coronavirus infections in humans as determined by enzyme-linked immunosorbent assay. *Infect Immun* 38(2): 419-23, 1982
- Macnaughton, M. R., D. Flowers and D. Isaacs. Diagnosis of human coronavirus infections in children using enzyme-linked immunosorbent assay. *J Med Virol* 11(4): 319-25, 1983
- Maggi, F., M. Pifferi, M. Vatteroni, C. Fornai, E. Tempestini, S. Anzilotti, L. Lanini, E. Andreoli, V. Ragazzo, M. Pistello, S. Specter and M. Bendinelli. Human metapneumovirus associated with respiratory tract infections in a 3-year study of nasal swabs from infants in Italy. *J Clin Microbiol* 41(7): 2987-91, 2003

-
- Manjarrez, M. E., D. P. Rosete, M. Rincon, J. Villalba, A. Cravioto and R. Cabrera. Comparative viral frequency in Mexican children under 5 years of age with and without upper respiratory symptoms. *J Med Microbiol* 52(Pt 7): 579-83, 2003
- Marin, J., D. Jeler-Kacar, V. Levstek and V. Macek. Persistence of viruses in upper respiratory tract of children with asthma. *J Infect* 41(1): 69-72, 2000
- McCannon, R. W. Natural history of respiratory tract infection patterns in basically healthy individuals. *Am J Dis Child* 122(3): 232-6, 1971
- McCormick, D. P., E. Lim-Melia, K. Saeed, C. D. Baldwin and T. Chonmaitree. Otitis media: can clinical findings predict bacterial or viral etiology? *Pediatr Infect Dis J* 19(3): 256-8, 2000
- McIntosh, K., R. K. Chao, H. E. Krause, R. Wasil, H. E. Mocega and M. A. Mufson. Coronavirus infection in acute lower respiratory tract disease of infants. *J Infect Dis* 130(5): 502-7, 1974
- McIntosh, K., A. Z. Kapikian, H. C. Turner, J. W. Hartley, R. H. Parrott and R. M. Chanock. Seroepidemiologic studies of coronavirus infection in adults and children. *Am J Epidemiol* 91(6): 585-92, 1970
- Meissner, H. Antiviral drugs for prophylaxis and treatment of influenza. *Pediatr Infect Dis J* 20(12): 1165-7, 2001
- Meissner, H. C., R. C. Welliver, S. A. Chartrand, D. R. Fulton, W. J. Rodriguez and J. R. Groothuis. Prevention of respiratory syncytial virus infection in high risk infants: consensus opinion on the role of immunoprophylaxis with respiratory syncytial virus hyperimmune globulin. *Pediatr Infect Dis J* 15(12): 1059-68, 1996
- Mertsola, J., T. Ziegler, O. Ruuskanen, T. Vanto, A. Koivikko and P. Halonen. Recurrent wheezy bronchitis and viral respiratory infections. *Arch Dis Child* 66(1): 124-9, 1991
- Mitchell, L. S., B. Taylor, W. Reimels, F. F. Barrett and J. P. Devincenzo. Adenovirus 7a: a community-acquired outbreak in a children's hospital. *Pediatr Infect Dis J* 19(10): 996-1000, 2000
- Monto, A. S. Studies of the community and family: acute respiratory illness and infection. *Epidemiol Rev* 16(2): 351-73, 1994
- Monto, A. S. Epidemiology of viral respiratory infections. *Am J Med* 112 Suppl 6A: 4S-12S, 2002
- Monto, A. S., A. M. Fendrick and M. W. Sarnes. Respiratory illness caused by picornavirus infection: a review of clinical outcomes. *Clin Ther* 23(10): 1615-27, 2001
- Monto, A. S., T. C. Shope, S. A. Schwartz and J. K. Albrecht. Intranasal interferon-alpha 2b for seasonal prophylaxis of respiratory infection. *J Infect Dis* 154(1): 128-33, 1986b
- Monto, A. S. and K. M. Sullivan. Acute respiratory illness in the community. Frequency of illness and the agents involved. *Epidemiol Infect* 110(1): 145-60, 1993
- Monto, A. S. and B. M. Ullman. Acute respiratory illness in an American community. The Tecumseh study. *JAMA* 227(2): 164-9, 1974
- Munoz, F. M. Influenza virus infection in infancy and early childhood. *Paediatr Respir Rev* 4(2): 99-104, 2003
- Myint, S., S. Johnston, G. Sanderson and H. Simpson. Evaluation of nested polymerase chain methods for the detection of human coronaviruses 229E and OC43. *Mol Cell Probes* 8(5): 357-64, 1994
- Mäkelä, M. J., T. Puhakka, O. Ruuskanen, M. Leinonen, P. Saikku, M. Kimpimäki, S. Blomqvist, T. Hyypiä and P. Arstila. Viruses and bacteria in the etiology of the common cold. *J Clin Microbiol* 36(2): 539-542, 1998

-
- Nafstad, P., J. A. Hagen, L. Oie, P. Magnus and J. J. Jaakkola. Day care centers and respiratory health. *Pediatrics* 103(4 Pt 1): 753-8, 1999
- Nurmi, T., E. Salminen and A. Pönkä. Infections and other illnesses of children in day-care centers in Helsinki. II: The economic losses. *Infection* 19(5): 331-5, 1991
- Oberste, M. S., K. Maher and M. A. Pallansch. Complete sequence of echovirus 23 and its relationship to echovirus 22 and other human enteroviruses. *Virus Res* 56(2): 217-23, 1998
- Pallansch, M. A. and R. P. Roos. Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. *Fields' Virology*. D. M. Knipe and P. M. Howley, Eds. Philadelphia, USA, Lippincott Williams & Wilkins. 1: 723-775, 2001
- Papadopoulos, N. G., P. J. Bates, P. G. Bardin, A. Papi, S. H. Leir, D. J. Fraenkel, J. Meyer, P. M. Lackie, G. Sanderson, S. T. Holgate and S. L. Johnston. Rhinoviruses infect the lower airways. *J Infect Dis* 181(6): 1875-84, 2000
- Paradise, J. L., H. E. Rockette, D. K. Colborn, B. S. Bernard, C. G. Smith, M. Kurs-Lasky and J. E. Janosky. Otitis media in 2253 Pittsburgh-area infants: prevalence and risk factors during the first two years of life. *Pediatrics* 99(3): 318-33, 1997
- Patel, J. A., B. Reisner, N. Vizirinia, M. Owen, T. Chonmaitree and V. Howie. Bacteriologic failure of amoxicillin-clavulanate in treatment of acute otitis media caused by nontypeable *Haemophilus influenzae*. *J Pediatr* 126(5 Pt 1): 799-806, 1995
- Peat, J. K., V. Keena, Z. Harakeh and G. Marks. Parental smoking and respiratory tract infections in children. *Paediatr Respir Rev* 2(3): 207-13, 2001
- Peiris, J. S., W. H. Tang, K. H. Chan, P. L. Khong, Y. Guan, Y. L. Lau and S. S. Chiu. Children with respiratory disease associated with metapneumovirus in Hong Kong. *Emerg Infect Dis* 9(6): 628-33, 2003a
- Peiris, J. S., K. Y. Yuen, A. D. Osterhaus and K. Stohr. The severe acute respiratory syndrome. *N Engl J Med* 349(25): 2431-41, 2003
- Peltola, H. Observations on the seasonal variation of the most common acute pediatric diseases in the Helsinki area (Finland). *J Community Health* 7(3): 159-70, 1982
- Pichichero, M. E., J. L. Green, A. B. Francis, S. M. Marsocci and M. L. Murphy. Outcomes after judicious antibiotic use for respiratory tract infections seen in a private pediatric practice. *Pediatrics* 105(4 Pt 1): 753-9, 2000
- Pinnock, C. B., R. M. Douglas and N. R. Badcock. Vitamin A status in children who are prone to respiratory tract infections. *Aust Paediatr J* 22(2): 95-9, 1986
- Pitkäranta, A., E. Arruda, H. Malmberg and F. G. Hayden. Detection of rhinovirus in sinus brushings of patients with acute community-acquired sinusitis by reverse transcription-PCR. *J Clin Microbiol* 35(7): 1791-1793, 1997
- Pitkäranta, A., J. Jero, E. Arruda, A. Virolainen and F. G. Hayden. Polymerase chain reaction-based detection of rhinovirus, respiratory syncytial virus, and coronavirus in otitis media with effusion. *J Pediatr* 133(3): 390-4, 1998
- Pitkäranta, A., P. Karma and T. Hovi. Deficiency in interferon production by leukocytes from children with recurrent respiratory infections. *Clin Diagn Virol* 1: 101-108, 1993
- Pitkäranta, A., J. Nokso-Koivisto, V. Jääntti, A. Takala, T. Kilpi and T. Hovi. Lowered yields of virus-induced interferon production in leukocyte cultures and risk of recurrent respiratory infections in children. *J Clin Virol* 14: 199-205, 1999

-
- Pitkäranta, A., M. Starck, S. Savolainen, T. Pöyry, I. Suomalainen, T. Hyypiä, O. Carpen and A. Vaheri. Rhinovirus RNA in the maxillary sinus epithelium of adult patients with acute sinusitis. *Clin Infect Dis* 33(6): 909-11, 2001
- Polack, F. P. and R. A. Karron. The future of respiratory syncytial virus vaccine development. *Pediatr Infect Dis J* 23(1): S65-S73, 2004
- Puhakka, H., E. Hagman, T. Heikkinen, P. Huovinen, J. Jero, P. Karma, M. Mäkelä, O. Ruuskanen and S. Sairanen. Äkillisen välikorvatulehduksen hoitosuositus [Recommended treatment of acute otitis]. *Duodecim* 115(19): 2155-61, 1999
- Putto, A. Febrile exudative tonsillitis: viral or streptococcal? *Pediatrics* 80(1): 6-12, 1987
- Putto-Laurila, A., J. Mertsola and O. Ruuskanen. Viral causes of tonsillitis and fever unresponsive to antibiotic therapy. *Pediatr Infect Dis J* 18(1): 71-2, 1999
- Rakes, G. P., E. Arruda, J. M. Ingram, G. E. Hoover, J. C. Zambrano, F. G. Hayden, T. A. Platts-Mills and P. W. Heymann. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care. IgE and eosinophil analyses. *Am J Respir Crit Care Med* 159(3): 785-90, 1999
- Rawlinson, W. D., Z. Waliuzzaman, I. W. Carter, Y. C. Belessis, K. M. Gilbert and J. R. Morton. Asthma exacerbations in children associated with rhinovirus but not human metapneumovirus infection. *J Infect Dis* 187(8): 1314-8, 2003
- Ray, C. G., C. J. Holberg, L. L. Minnich, Z. M. Shehab, A. L. Wright and L. M. Taussig. Acute lower respiratory illnesses during the first three years of life: potential roles for various etiologic agents. The Group Health Medical Associates. *Pediatr Infect Dis J* 12(1): 10-4, 1993
- Reed, G., P. H. Jewett, J. Thompson, S. Tollefson and P. F. Wright. Epidemiology and clinical impact of parainfluenza virus infections in otherwise healthy infants and young children < 5 years old. *J Infect Dis* 175(4): 807-13, 1997
- Rosen, F. S., M. D. Cooper and R. J. Wedgwood. The primary immunodeficiencies. *N Engl J Med* 333(7): 431-40, 1995
- Rosling, L. and M. Rosling. Pneumonia causes panic in Guangdong province. *BMJ* 326(7386): 416, 2003
- Ruohola, A., T. Heikkinen, M. Waris, T. Puhakka and O. Ruuskanen. Intranasal fluticasone propionate does not prevent acute otitis media during viral upper respiratory infection in children. *J Allergy Clin Immunol* 106(3): 467-71, 2000
- Ruuskanen, O., M. Arola, A. Putto-Laurila, J. Mertsola, O. Meurman, M. K. Viljanen and P. Halonen. Acute otitis media and respiratory virus infections. *Pediatr Infect Dis J* 8(2): 94-9, 1989
- Ryan, M. A., G. C. Gray, B. Smith, J. A. McKeegan, A. W. Hawksworth and M. D. Malasig. Large epidemic of respiratory illness due to adenovirus types 7 and 3 in healthy young adults. *Clin Infect Dis* 34(5): 577-82, 2002
- Räty, R., T. Ziegler and M. Kleemola. The value of virus serology in epidemiological studies of acute otitis media in children. *J Clin Virol* 29(4): 315-9, 2004
- Santti, J., T. Hyypia, L. Kinnunen and M. Salminen. Evidence of recombination among enteroviruses. *J Virol* 73(10): 8741-9, 1999
- Sarkkinen, H., O. Ruuskanen, O. Meurman, H. Puhakka, E. Virolainen and J. Eskola. Identification of respiratory virus antigens in middle ear fluids of children with acute otitis media. *J Infect Dis* 151(3): 444-8, 1985
- Savolainen, C., S. Blomqvist and T. Hovi. Human rhinoviruses. *Paediatr Respir Rev* 4(2): 91-8, 2003

-
- Schmidt, H. J. and R. J. Fink. Rhinovirus as a lower respiratory tract pathogen in infants. *Pediatr Infect Dis J* 10(9): 700-2, 1991
- Schwartz, R. H., A. Pitkäranta and B. Winther. Computed tomography imaging of the maxillary and ethmoid sinuses in children with short-duration purulent rhinorrhea. *Otolaryngol Head Neck Surg* 124(2): 160-3, 2001
- Sheikh, S., E. Allen, R. Shell, J. Hruschak, D. Iram, R. Castile and K. McCoy. Chronic aspiration without gastroesophageal reflux as a cause of chronic respiratory symptoms in neurologically normal infants. *Chest* 120(4): 1190-5, 2001
- Shulman, L. M., Y. Manor, R. Azar, R. Handsheer, A. Vonsover, E. Mendelson, S. Rothman, D. Hassin, T. Halmut, B. Abramovitz and N. Varsano. Identification of a new strain of fastidious enterovirus 70 as the causative agent of an outbreak of hemorrhagic conjunctivitis. *J Clin Microbiol* 35(8): 2145-9, 1997
- Simoes, E. A., J. R. Grootuis, D. A. Tristram, K. Alessi, M. V. Lehr, G. R. Siber and R. C. Welliver. Respiratory syncytial virus-enriched globulin for the prevention of acute otitis media in high risk children. *J Pediatr* 129(2): 214-9, 1996
- Simonsen, L., M. J. Clarke, G. D. Williamson, D. F. Stroup, N. H. Arden and L. B. Schonberger. The impact of influenza epidemics on mortality: introducing a severity index. *Am J Public Health* 87(12): 1944-50, 1997
- Sit, S. C., E. K. Yau, Y. Y. Lam, D. K. Ng, N. C. Fong, Y. W. Hui, W. F. Cheng, C. W. Leung and M. C. Chiu. A young infant with severe acute respiratory syndrome. *Pediatrics* 112(4): e257, 2003
- Söderström, M., B. Hovellius and K. Prellner. Children with recurrent respiratory tract infections tend to belong to families with health problems. *Acta Paediatr Scand* 80: 696-703, 1991a
- Söderström, M., B. Hovellius and K. Prellner. Respiratory tract infections in children with recurrent episodes as preschoolers. *Acta Paediatr Scand* 80(6-7): 688-95, 1991b
- Stanway, G., N. Kalkkinen, M. Roivainen, F. Ghazi, M. Khan, M. Smyth, O. Meurman and T. Hyypiä. Molecular and biological characteristics of echovirus 22, a representative of a new picornavirus group. *J Virol* 68(12): 8232-8, 1994
- Stockton, J., I. Stephenson, D. Fleming and M. Zambon. Human metapneumovirus as a cause of community-acquired respiratory illness. *Emerg Infect Dis* 8(9): 897-901, 2002
- Strikas, R. A., L. J. Anderson and R. A. Parker. Temporal and geographic patterns of isolates of nonpolio enterovirus in the United States, 1970-1983. *J Infect Dis* 153(2): 346-51, 1986
- Sunakorn, P., L. Chunchit, S. Niltawat, M. Wangweerawong and R. F. Jacobs. Epidemiology of acute respiratory infections in young children from Thailand. *Pediatr Infect Dis J* 9(12): 873-7, 1990
- Sung, B. S., T. Chonmaitree, L. D. Broemeling, M. J. Owen, J. A. Patel, D. C. Hedgpeth and V. M. Howie. Association of rhinovirus infection with poor bacteriologic outcome of bacterial-viral otitis media. *Clin Infect Dis* 17(1): 38-42, 1993
- Syrjänen, R. K., T. M. Kilpi, T. H. Kajjalainen, E. E. Herva and A. K. Takala. Nasopharyngeal carriage of *Streptococcus pneumoniae* in Finnish children younger than 2 years old. *J Infect Dis* 184(4): 451-9, 2001
- Thompson, D., G. Oster, L. J. McGarry and J. O. Klein. Management of otitis media among children in a large health insurance plan. *Pediatr Infect Dis J* 18(3): 239-44, 1999

-
- Tilles, J. G., J. O. Klein, R. L. Jao, J. E. Haslam, Jr., M. Feingold, S. S. Gellis and M. Finland. Acute otitis media in children. Serologic studies and attempts to isolate viruses and mycoplasmas from aspirated middle-ear fluids. *N Engl J Med* 277(12): 613-8, 1967
- Treanor, J. J. Influenza A and B viruses. *Viral infections of the respiratory tract*. P. F. Wright, Ed. New York, Marcel Dekker Inc. 127: 105-160, 1999
- Tsai, H. P., P. H. Kuo, C. C. Liu and J. R. Wang. Respiratory viral infections among pediatric inpatients and outpatients in Taiwan from 1997 to 1999. *J Clin Microbiol* 39(1): 111-8, 2001
- Tupasi, T. E., L. E. de Leon, S. Lupisan, C. U. Torres, Z. A. Leonor, E. S. Sunico, N. V. Mangubat, C. A. Miguel, F. Medalla, S. T. Tan and et al. Patterns of acute respiratory tract infection in children: a longitudinal study in a depressed community in Metro Manila. *Rev Infect Dis* 12 Suppl 8: S940-9, 1990
- Turner, R. B., M. T. Wecker, G. Pohl, T. J. Witek, E. McNally, R. St George, B. Winther and F. G. Hayden. Efficacy of tremacamra, a soluble intercellular adhesion molecule 1, for experimental rhinovirus infection: a randomized clinical trial. *JAMA* 281(19): 1797-804, 1999
- Ukkonen, P., T. Hovi, C.-H. v. Bonsdorff, P. Saikku and K. Penttinen. Age-specific prevalence of complement-fixing antibodies to sixteen viral antigens: a computer analysis of 58,500 patients covering a period of eight years. *J Med Virol* 13: 131-148, 1984
- Wairagkar, N. S., S. S. Gogate and A. S. Labhsetwar. Investigation of an epidemic of acute haemorrhagic conjunctivitis in Pune, India. *J Commun Dis* 31(1): 41-3, 1999
- Wald, E. R., B. Dashefsky, C. Byers, N. Guerra and F. Taylor. Frequency and severity of infections in day care. *J Pediatr* 112: 540-546, 1988
- Walsh, E. E. and B. S. Graham. Respiratory syncytial viruses. *Viral infections of the respiratory tract*. P. F. Wright, Ed. New York, Marcel Dekker Inc. 127: 161-204, 1999
- Vanecek, K. and A. Lehovcova. Interferon production in children with respiratory diseases. *Acta Paediatr Scand* 74(1): 118-21, 1985
- Waris, M. Pattern of respiratory syncytial virus epidemics in Finland: two-year cycles with alternating prevalence of groups A and B. *J Infect Dis* 163(3): 464-9, 1991
- Weigl, J. A., W. Puppe, B. Grondahl and H. J. Schmitt. Epidemiological investigation of nine respiratory pathogens in hospitalized children in Germany using multiplex reverse-transcriptase polymerase chain reaction. *Eur J Clin Microbiol Infect Dis* 19(5): 336-43, 2000
- Weigl, J. A., W. Puppe and H. J. Schmitt. Incidence of respiratory syncytial virus-positive hospitalizations in Germany. *Eur J Clin Microbiol Infect Dis* 20(7): 452-9, 2001
- Venuta, A., C. Spano, L. Laudizi, F. Bettelli, A. Beverelli and E. Turchetto. Essential fatty acids: the effects of dietary supplementation among children with recurrent respiratory infections. *J Int Med Res* 24(4): 325-30, 1996
- Vesa, S., M. Kleemola, S. Blomqvist, A. Takala, T. Kilpi and T. Hovi. Epidemiology of documented viral respiratory infections and acute otitis media in a cohort of children followed from two to twenty-four months of age. *Pediatr Infect Dis J* 20(6): 574-81., 2001
- Whitley, R. J., F. G. Hayden, K. S. Reisinger, N. Young, R. Dutkowski, D. Ipe, R. G. Mills and P. Ward. Oral oseltamivir treatment of influenza in children. *Pediatr Infect Dis J* 20(2): 127-33, 2001
- Viazov, S., F. Ratjen, R. Scheidhauer, M. Fiedler and M. Roggendorf. High prevalence of human metapneumovirus infection in young children and genetic heterogeneity of the viral isolates. *J Clin Microbiol* 41(7): 3043-5, 2003

-
- Williams, J. V., P. A. Harris, S. J. Tollefson, L. L. Halburnt-Rush, J. M. Pingsterhaus, K. M. Edwards, P. F. Wright and J. E. Crowe, Jr. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N Engl J Med* 350(5): 443-50, 2004
- Winther, B., F. G. Hayden, E. Arruda, R. Dutkowski, P. Ward and J. O. Hendley. Viral respiratory infection in schoolchildren: effects on middle ear pressure. *Pediatrics* 109(5): 826-32, 2002
- Viirolainen, A., P. Salo, J. Jero, P. Karma, J. Eskola and M. Leinonen. Comparison of PCR assay with bacterial culture for detecting *Streptococcus pneumoniae* in middle ear fluid of children with acute otitis media. *J Clin Microbiol* 32(11): 2667-70, 1994
- Woensel, v. J. B., W. M. van Aalderen and J. L. Kimpfen. Viral lower respiratory tract infection in infants and young children. *BMJ* 327(7405): 36-40, 2003
- Wright, P. F. Parainfluenza viruses. *Viral infections of the respiratory tract*. P. F. Wright, Ed. New York, Marcel Dekker Inc. 127: 205-222, 1999
- Yoshie, C. On the isolation of influenza virus from mid-ear discharge of influenza otitis media. *Jap J M Sc Biol* 8: 373-377, 1955
- Yun, B. Y., M. R. Kim, J. Y. Park, E. H. Choi, H. J. Lee and C. K. Yun. Viral etiology and epidemiology of acute lower respiratory tract infections in Korean children. *Pediatr Infect Dis J* 14(12): 1054-9, 1995
- Zambon, M. C. Epidemiology and pathogenesis of influenza. *J Antimicrob Chemother* 44 Suppl B: 3-9, 1999