MAJOR ARTICLE

Viral Upper Respiratory Tract Infection and Otitis Media Complication in Young Children

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(See the editorial commentary by Chavanet on pages 824)

Background. The common cold or upper respiratory infection (URI) is highly prevalent among young children and often results in otitis media (OM). The incidence and characteristics of OM complicating URI due to specific viruses have not been well studied.

Methods. We performed a prospective, longitudinal cohort study of 294 healthy children (age range, 6 months to 3 years). Each child was observed for 1 year to assess the occurrence of URI, acute OM (AOM), and OM with effusion (OME) complicating URI due to specific viruses.

Results. We documented 1295 URI episodes (5.06 episodes per child-year) and 440 AOM episodes (1.72 episodes per child-year). Virus studies were performed for 864 URI episodes; 63% were virus positive. Rhinovirus and adenovirus were most frequently detected during URI. The overall incidence of OM that complicated URI was 61%, including a 37% incidence of AOM and a 24% incidence of OME. Young age was the most important predictor of AOM that complicated URI. AOM occurred in approximately one-half of children with URI due to adenovirus, respiratory syncytial virus, or coronavirus and in approximately one-third of those with URI due to influenza virus, parainfluenza virus, enterovirus, or rhinovirus.

Conclusions. More than 60% of episodes of symptomatic URI among young children were complicated by AOM and/or OME. Young age and specific virus types were predictors of URI complicated by AOM. For young children, the strategy to prevent OM should involve prevention of viral URI. The strategy may be more effective if the priority is given to development of means to prevent URI associated with adenovirus and respiratory syncytial virus.

The common cold or upper respiratory infection (URI), a disease caused by a variety of viruses, is a universal illness. Particularly susceptible to URI are young children, especially those who attend day care centers [1–3]. URI in young children is often complicated by otitis media (OM) [4, 5]. The high prevalence of URI has made OM one of the most common diseases seen in pediatric practice and the emergency department [6, 7]. OM leads to widespread use of antibiotics and performance of otologic surgical procedures [8–10], thus draining public health resources worldwide [11–13]. Efforts to identify means to prevent OM are clearly needed.

OM is classified in 2 forms: acute OM (AOM), an acute symptomatic disease, and OM with effusion (OME), an asymptomatic disease involving fluid collection in the middle ear [14]. URI and AOM are closely linked; 29%–50% of all cases of URI develop into AOM [5, 15, 16], and a variety of viruses have been detected in nasopharynx and middle ear effusion specimens obtained from children with AOM [17, 18]. Data on URI that directly leads to new-onset OME are lacking. One way to prevent OM is to prevent URI in children. Because specific viruses may differ in their ability to induce OM, understanding the relative importance of URI-associated viruses will be useful in designing appropriate viral vaccines for OM prevention. Therefore, we performed a prospective study of young children to

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obtain epidemiologic information on URI and to determine the specific virus types associated with URI and their ability to induce AOM and OME.

METHODS

Study design and subjects. This was a prospective, longitudinal cohort study designed to capture all symptomatic episodes of URI that occurred in children during a 1-year period, to study the incidence and characteristics of URI that is complicated by OM. The study was performed at the University of Texas Medical Branch (Galveston, TX) and was approved by the Institutional Review Board; written informed consent was obtained for all subjects. Healthy children living in Galveston who had received medical care at the University of Texas Medical Branch were recruited from the primary care clinic and via advertisements in the local newspaper and at local day care centers. Children were enrolled at the ages of 6 months to 3 years. They could be asymptomatic or have had URI or AOM at the time of enrollment. Children with chronic medical problems or an anatomical or physiological defect of the ear or nasopharynx were excluded from the study. During the year, parents were asked to notify the study office as soon as the child began to have symptoms of a cold or URI (e.g., nasal congestion, rhinorrhea, cough, sore throat, or fever). Children were seen by a study physician as soon as possible after the onset and were observed a few days later to assess whether there were complications of OM; parents were compensated for time and travel. Study personnel also provided 2 home visits during weeks 2 and 3 of the URI to perform tympanometry. If the tympanogram findings remained abnormal after 3 weeks, testing was repeated every 2 weeks until the findings were normal or the next URI episode occurred. Parents were advised to bring the child for examination whenever they suspected the child to have any AOM symptom.

At each visit, information was collected on specific URI-related symptoms; tympanometry was performed, and the child's ears were examined using pneumatic otoscopy by trained investigators (T.C., K.R., and J.A.P.). OM was considered to have complicated URI if it occurred within 28 days after the onset of URI, unless new-onset URI occurred during this period; in that case, OM was considered to have complicated the most recent URI. AOM was defined as the acute onset of symptoms (fever, irritability, or earache), signs of tympanic membrane inflammation, and presence of fluid, as documented by pneumatic otoscopy and/or tympanometry. Children who received a diagnosis of AOM were treated on the basis of the standard of care [19]. OME was considered to have complicated URI if new fluid and/or an air-fluid bubble was visualized or if a new type B tympanogram result was obtained without signs of tympanic membrane inflammation. New middle ear effusion was defined as the presence of middle ear effusion without a type B tympanogram finding having been documented in the previous 30 days. Children with AOM and OME in each ear were counted as having AOM. The day of onset of OM during the course of URI was determined from day 1 (the first day of symptoms) to the first day of diagnosis.

In addition to parent's self-reported URI, the study personnel called the parents twice monthly to determine whether there were any current URI symptoms and occurrence of any URI or AOM episodes missed since prior contact. An extensive review of medical records was performed at the time of completion of each child's study participation. The University of Texas Medical Branch is the sole provider of pediatric health care in Galveston; diseases diagnosed and treated in our children are likely to be noted in our medical records. URI and AOM episodes not seen by the study group but captured from parent's interviews or from medical records were recorded as "missed episodes."

Virologic studies. Respiratory specimens were collected for virus studies at the initial URI visit and at subsequent visits only if AOM was diagnosed. Nasal swab specimens were collected for viral culture; nasopharyngeal secretions were collected for respiratory syncytial virus (RSV) antigen detection by EIA (performed only during RSV season) and for virus detection by molecular techniques (performed at the Medical College of Wisconsin; Milwaukee) on culture- and RSV-EIA–negative specimens [20, 21].

Real-time RT-PCR was performed with the PRISM 7300 Sequence Detection System (Applied Biosystems). Positive and negative results were determined with the autoanalysis software and were rechecked manually. For RNA viruses, the first stage conditions were 50°C for 30 min followed by 95°C for 15 min, 94°C for 15 s, and 60°C for 60 s for 45 cycles; for adenovirus, the conditions were 95°C for 15 min, 94°C for 15 s, 55°C for 30 s, and 72°C for 35 s for 45 cycles.

For adenovirus detection (hexon gene), the limit of detection was 1×10^2 $TCID_{50}$/mL. The limit of detection for coronavirus OC43/229E (N gene) and NL63 (N gene) were 10^{-2} $TCID_{50}$/mL and 10^{-1} $TCID_{50}$/mL, respectively; for rhinovirus (5′NTR gene), it was 10^{-2} $TCID_{50}$/mL; and for enterovirus (5′NTR), it was 10^{-2} $TCID_{50}$/mL. Analytical specificity was determined for each assay using American Type Culture Collection strains of adenovirus, enterovirus, OC43, 229E, rhinovirus, and influenza A virus; no cross-reactivity was detected. Validation using clinical samples demonstrated sensitivity of 95% (19 of 20 samples) versus tissue culture and 100% specificity (95% CI, 88%–100%) for adenovirus, sensitivity of 93% (13 of 14) and 97% specificity (95% CI, 83%–100%) for rhinovirus, and sensitivity of 75% (6 of 8) and 100% specificity (95% CI, 88%–100%) for enterovirus.

Table 1. Demographic characteristics and risk factor data at enrollment.

Characteristic	Value
No. of subjects	294
Age at enrollment, months	
Mean	13.7
Median	12
Male sex	150 (51)
Race or ethnicity	
White	173 (59)
Black	91 (31)
Biracial	23 (8)
Asian	7 (2)
Hispanic or Latino	164 (56)
Duration of observation	
12 months	201 (68)
6–11 months	40 (14)
Study dismissal for tympanostomy tube placement	8 (3)
Lost to follow-up before month 6 of study	45 (15)
Child care arrangement[a]	
Home	201 (69)
Home day care	23 (8)
Day care center	68 (23)
Breast-feeding[b]	139 (47)
Cigarette smoke exposure	102 (35)
Family history of otitis media	168 (57)
History of prior otitis media episodes[c]	133 (47)
Otitis-prone subjects[d]	13 (4)
Age-appropriate pneumococcal conjugate vaccination status	239 (81)

NOTE. Data are no. (%) of subjects, unless otherwise indicated.

[a] Information was available for 292 subjects. Of children who were cared for at home, 24% had no siblings.

[b] The median duration of breast-feeding was 16 weeks (range, 1 week to 1.4 years).

[c] Data were available for 284 subjects. The median number of prior episodes was 1 (range, 1–12).

[d] Defined as 3 episodes of otitis media in 6 consecutive months or as 4 episodes during 12 consecutive months.

RT-PCR with electronic microarray detection (NanoChip 400 system; Namogen) was performed for detection of RSV, parainfluenza types 1–3, and influenza A and B virus, as described elsewhere [21].

Statistical methods. The relationship of AOM or OME with a virus was analyzed using the general estimating equations approach, which treats the child as the unit of analysis while accounting for the multiple episodes of correlated data from each child. The repeated binary outcome of OM status was analyzed with a binomial distribution, logit link function, and AR(1) correlation structure. Analyses were conducted in the Genmod procedure in SAS software (SAS Institute) [22]. Rate ratios were calculated using Episheet 2001, Spreadsheets for the Analysis of Epidemiologic Data, by Rothman [23]. The episode-level culture- and molecular-positive rates of virus detection results were analyzed using Pearson χ^2 statistics.

RESULTS

Subjects. During the period January 2003 through March 2006, a total of 294 children were enrolled in the study; 46% entered the study in the first year of life, 42% in the second year, and 12% in the third year. Demographic characteristics and risk factor data are shown in table 1. Overall, the total duration of follow-up was 256 child-years; the median duration of follow-up per subject was 12 months (mean duration, 9.8 months).

Overall URI and OM episodes. A total of 1295 URI epi-

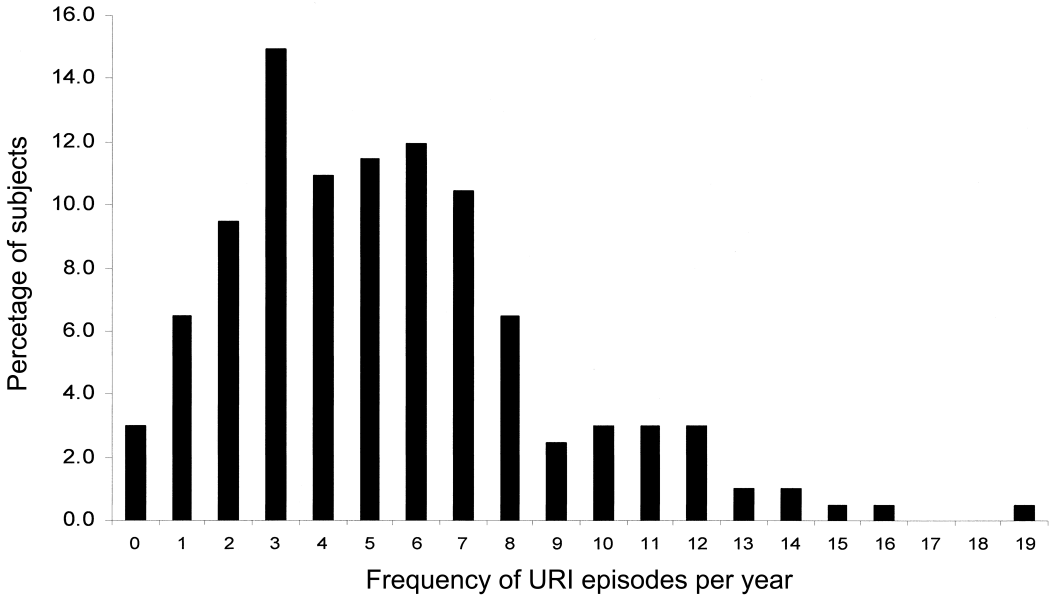

Figure 1. Number of upper respiratory infection (URI) episodes per year in 201 children observed by the study group for an entire 12-month period (mean number of episodes per child, 5.4; median, 5).

sodes were documented during 256 child-years (a rate of 5.06 episodes per child-year); the median age at the time of URI onset was 17.7 months. Another 26 AOM episodes without URI symptoms were documented. Patients accounting for 867 episodes (67%) of URI and for 6 episodes of AOM without URI were seen by the study group. Overall, there were 1839 study visits, 647 home or day care visits, and 4972 tympanograms obtained.

Of all URI episodes, 414 (32%) were complicated by AOM. The rate of AOM-complicated URI among episodes observed by the study group was 37%; the rate among missed URI episodes was 22%. With consideration of the 26 episodes of AOM that did not involve symptoms of URI, the total number of AOM episodes captured was 440 (1.72 episodes per child-year). The median age at the onset of AOM was 15.9 months.

Of 294 subjects, 201 subjects were observed for the entire 12 month-period; the remainder dropped out before 1 year had passed. The frequency of URI for each subject ranged from 0 to 19 cases per year (figure 1). Six subjects had no URIs (as determined by parent reports and chart reviews), and 7 experienced >12 URI episodes in 1 year. Frequencies of URI and AOM by age at enrollment and sex are shown in table 2.

Virologic findings. Of 867 URI episodes seen by the study group, specimens were collected during the first visit for 864;

Table 2. Frequencies of upper respiratory infection (URI) and acute otitis media (AOM) during a 1-year period for 201 children, by age and sex.

		No. or URI episodes per year			No. of AOM episodes per year		
Characteristic	No. of subjects	All children	Female children	Male children	All children	Female children	Male children
Age at enrollment							
6–11 months	89	5.34	5.58	5.17	1.91[a]	1.78	2.00[b]
12–23 months	85	5.58	6.21	4.76	1.67	1.65	1.70
24–35 months	27	5.07	5.07	5.08	1.07[a]	1.14	1.00[b]
All children	201	5.40	5.82	5.01	1.70	1.62	1.77
Total no. of episodes	…	1086	570	516	341	159	182

NOTE. Each child was observed for the entire 12-month period after enrollment. Children who attended a day care center, attended home day care, and were cared for at home had a mean of 6.3, 5.1, and 5.2 URI episodes per year, respectively (rate ratio for day care center vs. home, 1.2 [95% CI, 1.07–1.36; $P < .01$]; rate ratio for day care center vs. home day care, 1.24 [95% CI, 1.01–1.5; $P = .07$]).

[a] There was a statistically significant difference in the number of AOM episodes per year between subjects aged 6–11 months and those aged 24–35 months (rate ratio, 1.18; 95% CI, 1.2–2.6; $P < .005$).

[b] Rate ratio for subjects aged 6–11 months versus those aged 24–35 months, 2 (95% CI, 1.1–3.6; $P = .01$).

Table 3. Respiratory viruses detected during 864 episodes of upper respiratory infection.

Virus	No. (%) of episodes	
	Single or multiple viruses	Single agent
Adenovirus	208 (29)	114 (27)
Rhinovirus	176 (25)	112 (27)
Enterovirus	125 (18)	61 (14)
Coronavirus[a]	63 (9)	24 (6)
Parainfluenza virus types 1–3	54 (8)	42 (10)
Respiratory syncytial virus	42 (6)	38 (9)
Influenza A and B virus	35 (5)	29 (7)
Herpes simplex virus	5 (1)	2 (0.5)
Total	708	422

NOTE. A total of 708 respiratory viruses were detected from 547 (63%) of 864 URI episodes; 422 episodes (77%) were associated with a single virus.

[a] This included 14 cases involving coronavirus NL63; in 9 cases, this was the sole agent detected.

another 124 specimens were collected during the subsequent visits by patients with AOM. Overall, virologic studies were performed on 988 specimens obtained from 214 patients. Conventional viral assays detected viruses in 245 specimens (24.8%; positive viral culture results for 222 [22.4%] and negative culture but positive RSV EIA results for 23). During the period January 2003 through July 2006, a total of 600 specimens underwent molecular diagnostic testing; 587 of these were culture- and RSV antigen–negative specimens, and 359 (59.8%) were positive for $\geqslant$1 virus. Virus yield was 70% for specimens tested by conventional methods and by molecular diagnostics (when the results of conventional assays were negative). Specimens recovered from 4% of URI episodes were only tested by conventional assays. Overall, viruses were detected in specimens collected during 558 (64.6%) of 864 URI episodes; in 11 episodes, only cytomegalovirus was detected. Cytomegalovirus was also detected along with other viruses in 16 episodes. Because cytomegalovirus shedding may involve congenital or acquired infection, and because the virus may not be the cause of URI, cytomegalovirus data were excluded from the subsequent analyses. After the exclusion of cytomegalovirus data, 547 URI episodes were found to be associated with detection of respiratory viruses. Table 3 lists the 708 viruses detected in 547 (63%) of 864 URI episodes. One virus was detected in 422 episodes (77%), 2 viruses were detected in 92 episodes, 3 viruses were detected in 30 episodes, and 4 viruses were detected in 3 episodes.

AOM- and OME-complicated URI associated with specific viruses. For the 864 URI episodes observed by the study group that had available virus data, the incidence of URI complicated by AOM was 37% (319 of 864 episodes); 39% of AOM episodes were bilateral, 24% were right-side AOM, and 36% were left-side AOM. AOM was diagnosed on days 1–24 in the course of URI, with the peak occurring on days 3–5; the median time was day 4 (figure 2).

In 28 of 864 URI episodes, middle ear effusion (type B tympanogram) was already present within the 30 days before URI onset; these chronic middle ear effusions were excluded from consideration for new-onset OME. The incidence of OME-complicated URI was 24% (203 of 836 episodes); 23% of cases were right-side OME, 27% were left-side OME, and 51% were bilateral OME. The overall incidence of URI complicated by OM (i.e., AOM and OME) was 61%. The time of OME diagnosis in the course of URI is also shown in figure 2; median time was day 3.

Figure 3 illustrates the rate of OM-complicated URI for the 7 respiratory viruses; table 4 shows the rate of OM-complicated URI, the median day of diagnosis, and the age of the subjects at the time of onset of URI, AOM, and OME, by virus type. For each virus (except herpes simplex virus), the median age at onset of AOM was lower than that at onset of URI, suggesting that children who developed AOM were younger children. Coronavirus, RSV, and adenovirus were among the viruses associated with a higher rate of AOM. The episode data shown in table 4 were analyzed using the general estimating equations approach, which treated the child as the unit of analysis, thus accounting for the multiple correlated episodes in each child. The overall model indicated that age ($P < .001$) was the strongest predictor of AOM development after URI, followed by virus type ($P = .05$), controlling for sex ($P = .19$), race ($P = .92$), and ethnicity ($P = .75$). For age, the OR was 0.96 (95% CI, 0.94–0.98), meaning that, for each additional month of age in the URI episode, the chances of developing AOM decreased by 4%. Table 5 compares the differences among the rates of AOM associated with specific viruses. For OME outcome, general estimating equations data revealed statistical significance only for age (OR, 0.98; 95% CI, 0.96–0.99; $P = .03$).

AOM rate determined by virus diagnostic methods. Because molecular assays are more sensitive than conventional viral diagnostic assays, which likely detect virus only when it is present in larger quantities (i.e., a high viral load), we compared the rate of AOM associated with specific viruses detected by different methods (table 6). For each virus, the AOM rate was higher in cases diagnosed with viral culture, compared with those diagnosed with molecular assays. Overall, cases diagnosed with culture were associated with a higher rate of AOM, compared with cases diagnosed with molecular methods ($P = .001$).

DISCUSSION

We have demonstrated a high susceptibility of young children to URI and a strikingly high rate of OM complications. Our statistical model identified the age of the child as the most important factor of AOM-complicated URI. The 2 viruses most

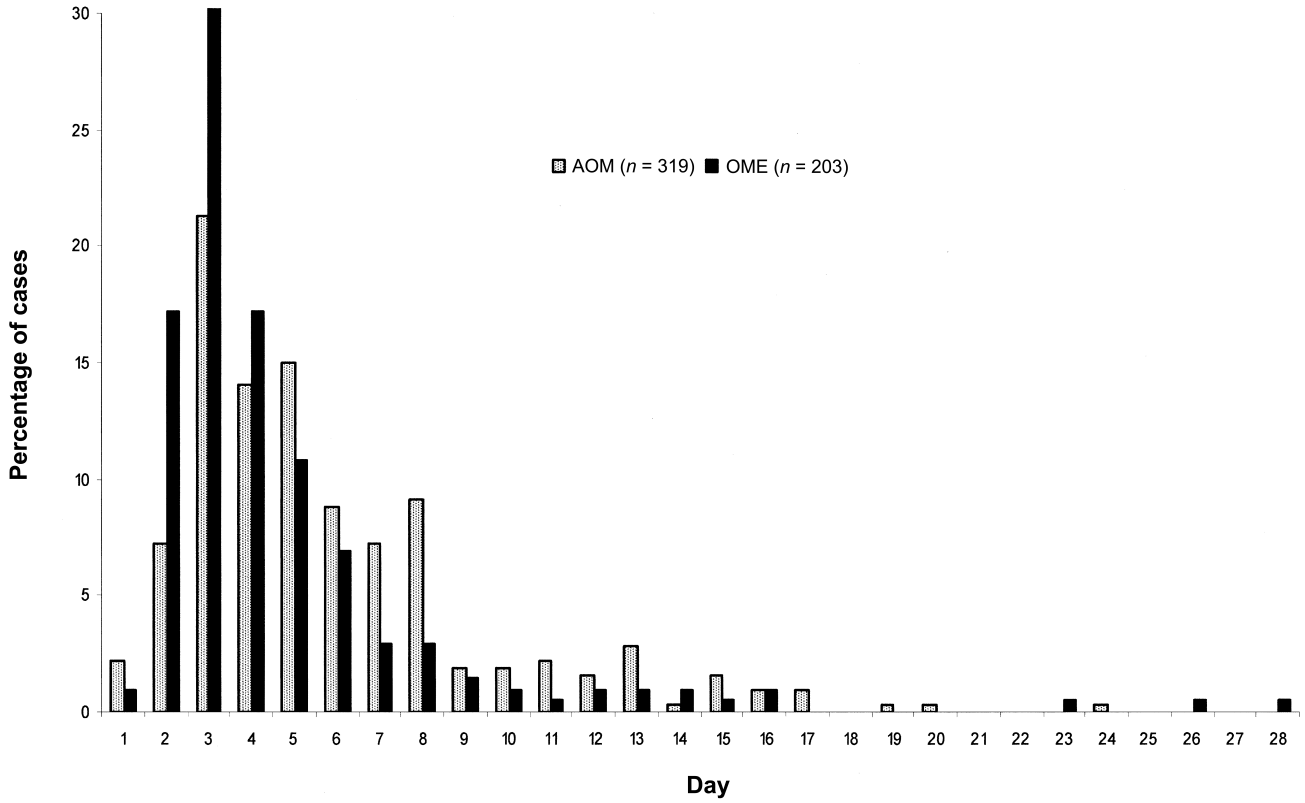

Figure 2. Day of diagnosis of acute otitis media (AOM) and otitis media with effusion (OME) in the course of upper respiratory infection

commonly detected during URI were rhinovirus and adenovirus. Although adenovirus was associated with high rate of AOM-complicated URI, rhinovirus was associated with lower rate than that of coronavirus, RSV, and adenovirus. Our data emphasize the close relationship between viral URI and OM and suggest that one strategy to reduce OM incidence is to prevent viral URI in young children.

The relative role of specific virus types in AOM reported in previous studies has been inconclusive, because different virus detection methods were used. Henderson et al. [24] used viral culture and reported that RSV, adenovirus, and influenza virus were closely associated with AOM; the incidence of AOM associated with rhinovirus URI was the lowest. Ruuskanen et al. [25] used conventional viral assays and reported similar results. Vesa et al. [26] found that URI associated with RSV and rhinovirus had a higher rate of AOM than did URI associated with adenovirus, but these authors used PCR alone for detection of rhinovirus and antigen detection for detection of other viruses. Pitkaranta et al. [27] used RT-PCR and reported that rhinovirus (35%) was the most common virus found in cases of nasopharyngeal secretions and/or middle ear effusion in children with AOM (the rates for RSV and coronavirus were 28% and 17%, respectively), although the children in that study were relatively old (median age, 30 months). We used more-comprehensive diagnostic methods than other researchers and more often detected adenovirus during URI; the virus was also associated with the highest rate of AOM-complicated URI. We were surprised that RSV-associated URI was not diagnosed more frequently; this could be because there was an unusually low prevalence of RSV in our community during the study period or because young children with RSV infection tended to have lower respiratory tract disease rather than URI. Nevertheless, the rate of RSV-associated URI complicated by AOM was among one of the highest; this is consistent with findings from previous reports [24, 28, 29]. Our data together suggest that prevention of adenovirus- and RSV-associated URI—if and when possible—has the potential to make a significant impact on the incidence of AOM.

Because URI is self-limiting, viral diagnosis is not clinically indicated. Research studies of viral etiology of URI that use a variety of viral diagnostic methods, including molecular techniques, have provided a virus yield of 42%–73% [26, 30, 31]. Virus yield depends on many factors, including sensitivity of the technique, specificity of the primers, and the number of viruses targeted. New viruses have recently been discovered as causes of URI and/or OM: human metapneumovirus, bocavirus, and coronavirus NL-63 [32–34]. The limited amount of samples and the high cost prohibited us from testing for all

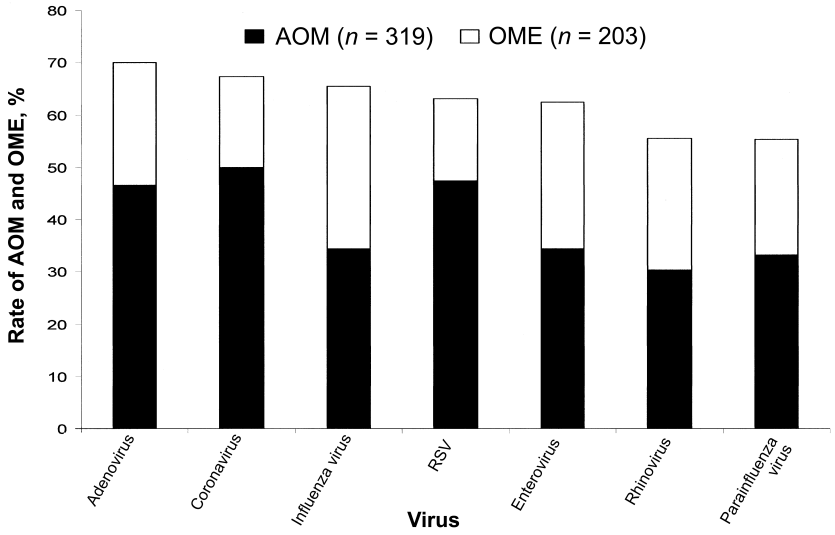

Figure 3. Rate of acute otitis media (AOM) and otitis media with effusion (OME) by upper respiratory infection–associated virus, for all virus detection methods combined. RSV, respiratory syncytial virus.

viruses. We did not test for human metapneumovirus and bocavirus, which could have lowered the virus yield by 5%–10% [32, 34]. Our virus types and yield are comparable to those that have been reported elsewhere [26, 30, 31], although the epidemiology of respiratory viral infection varies by geographical location and from year to year.

Recently, investigators found viruses in respiratory specimens obtained from children with no symptoms [31]; rhinovirus has also been found to have a prolonged presence in the respiratory tract [35, 36]. Because viruses are intracellular pathogens, these cases constitute asymptomatic infections. We studied only symptomatic URI and assumed a cause-and-effect relationship.

Table 4. Rates of acute otitis media (AOM) and new-onset otitis media with effusion (OME) following upper respiratory infection, by respiratory viruses and median age.

	URI		AOM			OME			
Virus	No. of episodes	Age, median months[a]	Rate, %[b]	Median day[c]	Age, median months[a]	Rate, %[b]	Median day[c]	Age, median months[a]	Total rate of otitis media[b]
Adenovirus	114[d]	18	46.5	5	16	23.6	6	19	70.1
Rhinovirus	112[e]	18	30.4	5	16	25.2	5	16	55.6
Enterovirus	61[f]	17	34.4	5	16	28.1	4	15	62.5
Parainfluenza	42	17	33.3	4	16	22.0	4	13	55.3
RSV	38	20	47.4	5	16	15.8	8	18	63.2
Influenza virus	29[g]	24	34.5	4	16	31.0	4	27	65.5
Coronavirus	24	19	50.0	6	16	17.4	5	19	67.4
HSV	2	14	50.0	15	16	0.0	0	0	50.0
Combined viruses	125	18	36.8	5	16	22.1	5	16	58.9
No virus	317	17	34.7	5	16	25.7	5	16	60.4
Total	864	18	36.9	5	16	24.3	5	16	61.2

NOTE. Episodes associated with specific viruses included a single virus per episode; otherwise, they are included as combined infections. Statistical analyses was performed using the general estimating equations model (see Methods). HSV, herpes simplex virus; RSV, respiratory syncytial virus.

[a] Median age at time of onset of URI, AOM, or OME.
[b] Rate of AOM- or OME-complicated URI caused by a specific virus.
[c] Day of otitis media during the course of URI.
[d] For 30 patients (26%), the same virus was detected in prior episodes 7–89 days previously.
[e] For 7 patients (6%), the same virus was detected in prior episodes 14–50 days previously.
[f] For 10 patients (16%), the same virus was detected in prior episodes 8–73 days previously.
[g] In 1 case (3%), the same virus was detected in prior episodes 39 days previously.

Table 5. ORs for pair-wise comparisons of the rate of upper respiratory infection complicated by acute otitis media, by specific virus.

Virus	OR (95% CI)	*P*
Coronavirus vs. rhinovirus	3.0 (1.32–6.98)	.009
Coronavirus vs. negative	2.8 (1.20–6.61)	.018
Coronavirus vs. enterovirus	2.5 (1.03–6.07)	.043
RSV vs. rhinovirus	2.1 (1.11–3.92)	.023
RSV vs. negative	1.9 (1.11–3.34)	.019
Adenovirus vs. rhinovirus	1.8 (1.06–3.10)	.029
Adenovirus vs. negative	1.7 (1.09–2.59)	.019

NOTE. Only statistically significant comparisons are shown. RSV, respiratory syncytial virus.

We only compared the rate of OM in URI episodes associated with single virus; of these, 11% revealed the same virus as in previous episodes (table 4). In addition, 125 (23%) of our 547 virus-positive samples contained ⩾2 viruses. Other researchers have reported rates of dual- or multiple-virus infection of 5%–20% [26, 30, 31, 37]; our relatively high rate could be associated with the more-comprehensive assays that we used or with frequent collection of samples from children with recurrent URI. In any event, it was possible that the molecular assays may have also detected some of the virus associated with previous URI episodes. The role of prolonged presence of viruses in the respiratory tract and that of dual- or multiple-virus infections in OM requires further investigations.

Positive viral culture results are associated with detection of live virus and are a strong indication of the cause-and-effect relationship (i.e., a viral cause of current symptoms). The findings of high rates of AOM associated with isolation of adenovirus and RSV help confirm the significance of these viruses in AOM-complicated URI. We have also found that, for every virus that both culture and molecular assays detected, the rate of AOM was higher for cases detected by culture. Tissue culture is less sensitive than molecular assays and probably detects virus only in larger quantities (i.e., a high viral load). Therefore, our finding also suggests the role of high viral load in increasing URI severity. Correlations between virus concentrations and elevated levels of cytokines/inflammatory mediators (e.g., IL-6, TNF-α, IFN-γ, IL-1, IL-8, and macrophage inflammatory protein–1α), and disease severity have been shown previously in respiratory virus infections [38–44]. It is likely that, in our cases, higher viral loads generated higher degrees of inflammation, which may have worsened the eustachian tube function, leading to OM complication.

In conclusion, we found a high prevalence of symptomatic viral URI among young children, and >60% of cases were complicated by AOM and/or OME. The risk of AOM development was associated with young age, specific URI-associated viruses, and conventional methods of viral detection. The role of persistence of viral nucleic acids in respiratory secretions obtained from children with symptomatic URI requires further investigations.

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References

1. Fox JP, Hall CE, Cooney MK, et al. The Seattle virus watch. II. Objectives, study population and its observation, data processing and summary of illness. Am J Epidemiol **1972**; 96:270–85.

Table 6. Rates of acute otitis media (AOM) associated with specific viruses, by virus detection method.

	All methods		Culture-positive cases		Molecular study–positive cases		RSV EIA–positive cases	
Virus	No. of cases	AOM rate, %	No. of cases	AOM rate, %	No. of cases	AOM rate, %	No. of cases	AOM rate, %
Adenovirus	114	46.5	30	63.3	84	40.4	…	…
Rhinovirus	112	30.4	52	42.3	60	20.0	…	…
Enterovirus	61	34.4	24	41.7	37	29.7	…	…
Parainfluenza	42	33.3	30	36.7	12	25.0	…	…
RSV	38	47.4	12	58.3	4	0.0	22	50.0
Influenza	29	34.5	25	40.0	4	0.0	…	…

NOTE. Cases diagnosed by culture were associated with higher rate of AOM-complicated URI, compared with cases diagnosed by molecular methods ($P = .001$, by Pearson χ^2 test). RSV, respiratory syncytial virus.

2. Monto AS, Ullman BM. Acute respiratory illness in an American community: the Tecumseh study. JAMA **1974**; 227:164–9.
3. Stahlberg MR. The influence of form of day care on occurrence of acute respiratory tract infections among young children. Acta Paediatr Scand **1980**; 282(Suppl 1):1–87.
4. Heikkinen T. Role of viruses in the pathogenesis of acute otitis media. Pediatr Infect Dis J **2000**; 19:17–23.
5. Winther B, Doyle WJ, Alper CM. A high prevalence of new onset otitis media during parent diagnosed common colds. Int J Pediatr Otorhinolaryngol **2006**; 70:1725–30.
6. Fried VM, Mukuc DM, Rooks RN. Ambulatory health care visits by children: principal diagnosis and place of visits. Vital Health Stat **1998**; 13:1–23.
7. Stussman BJ. National hospital ambulatory medical care survey: 1993 emergency department summary. Atlanta: Vital Health Statistics of the Centers for Disease Control and Prevention/National Center for Health Statistics **1996**; 271:1–15.
8. McCaig LF, Besser RE, Hughes JM. Trends in antimicrobial prescribing rates for children and adolescents. JAMA **2002**; 287:3096–102.
9. Teele DW, Klein JO, Chase C, Menyuk P, Rosner BA; Greater Boston Otitis Media Study Group. Otitis media in infancy and intellectual ability, school achievement, speech, and language at age 7 years. J Infect Dis **1990**; 162:685–94.
10. Postma DS, Poole MD, Wu SM, Tober R. The impact of day care on ventilation tube insertion. Int J Pediatr Otorhinolaryngol **1997**; 41: 253–62.
11. Stool SE, Field MJ. The impact of otitis media. Pediatr Infect Dis J **1989**; 8:11–4.
12. Gates GA. Cost-effectiveness considerations in otitis media treatment. Otolaryngol Head Neck Surg **1996**; 114:525–30.
13. Joki-Erkkila VP, Pukander J, Laippala P. Alteration of clinical picture and treatment of pediatric acute otitis media over the past two decades. Int J Pediatr Otorhinolaryngol **2000**; 55:197–201.
14. Bluestone CD, Klein JO. Definitions, terminology and classification. In: Otitis media in infants and children. 4th ed. Hamilton, Ontario: BC Decker, **2007**:1–19.
15. Heikkinen T. Temporal development of acute otitis media during upper respiratory tract infection. Pediatr Infect Dis J **1994**; 13:659–61
16. Koivunen P, Kontiokari T, Neimelä M, et al. Time to development of acute otitis media during an upper respiratory tract infection in children. Pediatr Infect Dis J **1999**; 18:303–5.
17. Chonmaitree T. Viral and bacterial interaction in acute otitis media. Pediatr Infect Dis J **2000**; 19(Suppl 5):24–30.
18. Ruohola A, Meurman O, Nikkari S, et al. Microbiology of acute otitis media in children with tympanostomy tubes: prevalences of bacteria and viruses. Clin Infect Dis **2006**; 43:1417–22.
19. Subcommittee on Management of Acute Otitis Media, American Academy of Pediatrics and American Academy of Family Physicians. Diagnosis and management of acute otitis media. Pediatrics **2004**; 113: 1451–65.
20. Fan J, Henrickson KJ, Savatski LL. Rapid simultaneous diagnosis of infections with respiratory syncytial viruses A and B, influenza viruses A and B, and human parainfluenza virus types 1, 2, and 3 by multiplex quantitative reverse transcription–polymerase chain reaction–enzyme hybridization assay (Hexaplex). Clin Infect Dis **1998**; 26:1397–402.
21. Henrickson KJ, Kraft A, Shaw J, Canter D. Comparison of electronic microarray (NGEN RVA) to enzyme hybridization assay (Hexaplex) for multiplex RT-PCR detection of common respiratory viruses in children. Clinical Microbiology Newsletter **2007**; 29:113–9.
22. SAS Institute. SAS/STAT 9.1 user's guide. Cary, NC: SAS Institute, **2004.**
23. Rothman K. Spreadsheets for the analysis of epidemiological data 2004. Available at: http://members.aol.com/krothman/episheet.xls.
24. Henderson FW, Collier AM, Sanyal MA, et al. A longitudinal study of respiratory viruses and bacteria in the etiology of acute otitis media with effusion. N Engl J Med **1982**; 306:1377–83.
25. Ruuskanen O, Arola M, Putto-Laurila A, et al. Acute otitis media and respiratory virus infections. Pediatr Infect Dis J **1989**; 8:94–9.
26. Vesa S, Kleemola M, Blomqvist S, Takala A, Kilpi T, Hovi T. 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 **2001**; 20:574–81.
27. Pitkaranta A, Virolainen A, Jero J, Arruda E, Hayden FG. Detection of rhinovirus, respiratory syncytial virus, and coronavirus infections in acute otitis media by reverse transcriptase polymerase chain reaction. Pediatrics **1998**; 102:291–5.
28. Heikkinen T, Thint M, Chonmaitree T. Prevalence of various respiratory viruses in the middle ear during acute otitis media. N Engl J Med **1999**; 340:260–4.
29. Patel JA, Nguyen D, Revai K, Chonmaitree T. Role of respiratory syncytial virus in acute otitis media: implications for vaccine development. Vaccine **2007**; 25:1683–9.
30. Makela MJ, Puhakka T, Ruuskanen O, et al. Viruses and bacteria in the etiology of the common cold. J Clin Microbiol **1998**; 36:539–42.
31. Winther B, Alper CM, Mandel EM, Doyle WJ, Hendley JO. Temporal relationships between colds, upper respiratory viruses detected by polymerase chain reaction, and otitis media in young children followed through a typical cold season. Pediatrics **2007**; 119:1069–75.
32. Williams JV, Wang CK, Yang CF. et al. The role of human metapneumovirus in upper respiratory tract infections in children: a 20-year experience. J Infect Dis **2006**; 193:387–95.
33. Williams JV, Tollefson SJ, Nair S, Chonmaitree T. Association of human metapneumovirus with acute otitis media. Int J Pediatr Otorhinolaryngol **2006**; 70:1189–93.
34. Kesebir D, Vazquez M, Weibel C. et al. Human bocavirus infection in young children in the United States: molecular epidemiological profile and clinical characteristics of a newly emerging respiratory virus. J Infect Dis **2006**; 194:1276–82.
35. Winther B, Hayden FG, Hendley JO. Picornavirus infections in children diagnosed by RT-PCR during longitudinal surveillance with weekly sampling: association with symptomatic illness and effect of season. J Med Virol **2006**; 78:644–50.
36. Rihkanen H, Carpen O, Roivainen M, Vaheri A, Pitkaranta A. Rhinovirus in adenoid tissue. Int J Pediatr Otorhinolaryngol **2004**; 68: 903–8.
37. Legg JP, Warner JA, Johnston SL, Warner JO. Frequency of detection of picornaviruses and seven other respiratory pathogens in infants. Pediatr Infect Dis J **2005**; 24:611–6.
38. Sheeran P, Jafri H, Carubelli C, et al. Elevated cytokine concentrations in the nasopharyngeal and tracheal secretions of children with respiratory syncytial virus disease. Pediatr Infect Dis J **1999**; 18:115–22.
39. Fritz R, Hayden F, Calfee D, et al. Nasal cytokine and chemokine responses in experimental influenza A virus infection: results of a placebo-controlled trial of intravenous zanamivir treatment. J Infect Dis **1999**; 180:586–93.
40. Hornsleth A, Larsen LL. Cytokines and chemokines in respiratory secretion and severity of disease in infants with respiratory syncytial virus infection. J Clin Virol **2001**; 21:163–70.
41. Hayden F, Fritz RS, Lobo MC, et al. Local and systemic cytokine responses during experimental human influenza A virus infection. J Clin Invest **1998**; 101:643–9.
42. Mistchenko AS, Diez, RA, Mariani AL, et al. Cytokines in adenoviral disease in children: association of interleukin-6, interleukin-8, and tumor necrosis factor alpha levels with clinical outcome. J Pediatr **1994**; 124:714–20.
43. Proud D, Gwaltney JM, Hendley O, Dinarello CA, Gills S, Schleimer RP. Increased levels of interleukin-1 are detected in nasal secretions of volunteers during experimental rhinovirus colds. J Infect Dis **1994**; 169:1007–13.
44. Turner RB, Weingand KW, Chyon-Hwa Y, Leedy DW. Association between interleukin-8 concentration in nasal secretions and severity of symptoms of experimental rhinovirus colds. Clin Infect Dis **1998**; 26: 840–6.