OBJECTIVES. The purpose of this work was to assess the impact of recently described human metapneumovirus and human coronavirus NL63 compared with other respiratory viruses by using sensitive molecular techniques in a cohort of healthy preschool-aged children. We also aimed to assess the use of parent collection to obtain an adequate respiratory specimen from acutely unwell children in the community.

PATIENTS AND METHODS. The community epidemiology and burden of human metapneumovirus and other respiratory viruses (influenza A, influenza B, respiratory syncytial virus, parainfluenza viruses, adenoviruses, and picornaviruses) were examined in a cohort of 234 preschool-aged children from Melbourne, Australia, over a 12-month period by using polymerase chain reaction testing. Parents collected a daily symptom diary for the duration of the study and were taught to collect a combined nose-throat swab and complete an impact diary when the study child had an acute respiratory illness.

RESULTS. The average incidence of acute respiratory illness was 0.48 per child-month for the duration of the study, with a winter peak. Of 543 illnesses with ≥1 specimen returned, 33 were positive for human metapneumovirus (6.1%) and 18 for human coronavirus NL63 (3.3%). Of all of the viruses for which we tested, human metapneumovirus and human coronavirus NL63 were most strongly linked to child care attendance, occurring in 82% and 78% of infected children, respectively. Picornaviruses were the most commonly identified virus group (269 [49.5%]). Influenza virus and adenovirus illnesses had the greatest impact, with fever in more than three quarters and requiring, on average, >1 local doctor visit per illness.

CONCLUSIONS. Recently identified human metapneumovirus and human coronavirus NL63 are important pathogens in community-based illness in children, particularly in those who attend child care. Picornaviruses were detected in half of the nose-throat swabs collected during acute respiratory illness in children but resulted in milder illnesses; influenza and adenovirus caused the highest-impact illnesses. The use of parent-collected specimens should be considered for additional community-based epidemiologic studies and vaccine trials.
A CUTE RESPIRATORY ILLNESSES (ARI s) in children are common, often caused by viruses, and can be serious or life threatening. Available information for viral ARIs in children is largely based on findings from those with illnesses severe enough to warrant hospitalization or on community-based studies performed previously without the use of sensitive molecular techniques. This means our current understanding not only lacks detail of the community epidemiology of recently identified pathogens, such as human metapneumovirus (hMPV) and human coronavirus (hCoV)-NL63, but also of other viruses for which traditional, nonmolecular techniques are less sensitive.

Since their initial discovery, hMPV and hCoV-NL63 have been identified with varying prevalence in specimens from healthy and compromised children and adults presenting to the hospital with mild or severe respiratory illnesses. However, information about community incidence and severity using molecular techniques for these and other respiratory viruses is lacking.

New respiratory virus vaccines are now in clinical trials with human subjects and there is promise of other novel therapeutic options. Large-scale public health interventions to limit the impact of a significant proportion of respiratory viral infections, particularly in childhood, are now a real possibility. With this study we sought to describe the population epidemiology and impact of common respiratory viruses in children. This information is required to inform public health prevention strategies and to fill the gap in the literature around community-based data derived using molecular techniques. We also sought to compare the relative importance of the recently discovered hMPV and hCoV-NL63 to other viruses.

PATIENTS AND METHODS

Study Cohort

This study was conducted in the greater Melbourne area. Data and specimen collection commenced on January 17, 2003, and concluded on January 31, 2004. Recruitment and enrollment of children were progressive, with the last child enrolled on November 5, 2003. Recruitment took place through maternal and child health nurses from 26 local councils; by placing advertising material at child care and playgroup centers; and through bulletin boards and staff e-mail lists at the Royal Children’s and the Royal Women’s Hospitals in Melbourne. This was a dynamic cohort with subjects able to temporarily leave the study (such as during family holidays) and rejoin at a later date.

We enrolled 1 child <5 years of age as the primary study subject from each participating family. Where >1 child per household was eligible, we enrolled the child whose birthday fell next. Screening and enrollment visits were conducted by a research assistant, where study procedures were explained. Risk-factor and household demographic data were collected at the enrollment visit and updated during the study. Children were eligible for enrollment and continuing participation if they were generally healthy and did not have any specific condition that predisposed them to acquiring ARIs or more severe illness with ARIs, such as being born at <36 weeks’ gestation; a chronic heart or respiratory disorder, including a diagnosis of asthma; or another chronic health problem, such as diabetes, kidney disease, or an immune disorder. The study was approved by the Royal Children’s Hospital Ethics in Human Research Committee, and written informed consent was obtained from parents or guardians before participation.

Illness Identification and Evaluation

Parents kept a daily symptom diary for the study child based on that used in the study by Belshe et al to assess the efficacy of the live, cold-adapted, intranasal, trivalent influenza vaccine in children. Symptoms were classified as category A (fever, wheezing, shortness of breath, pulmonary congestion or moist cough, pneumonia, or ear infection) and category B (runny nose or nasal congestion, sore throat, cough, muscle aches, chills, headache, irritability, decreased activity or lassitude or weakness, or vomiting). An ARI of interest required ≥1 category A or 2 category B symptoms on a single day. Other than pneumonia, which we asked parents to record only if supported by a health care professional’s diagnosis, no illness or symptom details were validated by study staff or health care professionals. A new ARI could not commence unless there were ≥3 symptom-free days since the end of the previous ARI. This meant an ARI could contain no more than 2 consecutive symptom-free days.

When a new ARI occurred, parents were asked to collect a combined nose and throat swab and to complete an impact diary detailing resources used in illness management. Parents were taught specimen collection at the enrollment visit, and simple instructions were left for reference. Parents could seek telephone guidance, and research staff were available to visit the home to assist with or perform specimen collection, where required. Separate swabs for the nose and throat were pooled into viral transport media and transported via courier in a small polystyrene transport container with an ice brick to the Victorian Infectious Diseases Reference Laboratory (VIDRL). If collected overnight or on a weekend, specimens were stored in a biohazard bag in the household refrigerator and collected the next working day.

Study families were contacted by telephone or e-mail regularly, every 2 to 3 weeks, to encourage compliance with study procedures, diary return, and to assess continuing eligibility. Subsequent respiratory illnesses in household contacts were recorded where onset was...
within 7 days of ARI symptoms in the study child. Families were informed of virus testing results by mail when they became available.

Laboratory Studies
Specimens were tested for respiratory viruses by polymerase chain reaction (PCR) testing at VIDRL the same day or the next working day after arrival, and original specimens and nucleic acid extracts were then stored at $-70^\circ$C. The testing and validation of a diagnostic multiplex PCR for detecting respiratory viruses by VIDRL has been published elsewhere. These assays were used to test specimens for influenza A virus (H1 and H3 subtypes), influenza B virus, adenoviruses, respiratory syncytial virus (RSV), picornaviruses (enteroviruses and rhinoviruses), and parainfluenza virus (PIV) types 1, 2, and 3, corresponding with tubes 1, 2, and 3, as described previously.

All of the specimens were transferred on dry ice at study completion to the Queensland Pediatric Infectious Diseases Laboratory at the Royal Children’s Hospital, Brisbane, for hMPV PCR testing using a real-time assay and hCoV-NL63 PCR testing using 2 nested assays, all as described previously.

Statistical Analysis
The total number of ARIs and incidence rates with 95% confidence intervals (CIs) are presented. In calculating incidence rates, only at-risk child-days were included in the denominator, with the removal of those days contained within an ARI and the 3 days subsequent. Stratum-specific rates and univariate incident rate ratios by age, child care attendance as categorized by the Australian Bureau of Statistics, month, and other risk factors were also calculated. All of the calculations were performed using Stata 9.2 for Windows (Stata Corp, College Station, TX).

RESULTS
Study Cohort
A total of 234 under 5 years of age were enrolled progressively on the study. We received 56 397 child-days of data (82.5%) from a maximum possible 68 400 days from 229 children. No daily data were received from 5 children (2.1%).

The greatest representation (28%) of child-days came from children aged between 1 and 2 years (Table 1). Using the child’s age on each day of data submission, the mean age of contributing child time was 29.0 months. Similar numbers of child-days were contributed by boys and girls. Children not in any child care accounted for approximately one third of enrolled subjects and person time (Table 1).

Acute Respiratory Infections
A total of 730 ARIs were identified. Of the 56 397 child-days of data available, 8926 (15.8%) were contained within an ARI (mean duration: 12.2 days), and 2138 days were subsequent not-at-risk days, leaving 46 063 at-risk child-days (including the first day of each ARI), or 1513 child-months, for rate calculations. This gives an incidence rate of 0.48 ARIs per child-month (95% CI: 0.45–0.52) or 5.8 ARIs per child year (95% CI: 5.4–6.2).

The peak rate of ARIs was in June at 0.87 ARIs per child-month (Fig 1). The proportion of specimens positive for any virus was highest in October (84%) and lowest in August (62%; Fig 1). Children aged between 1 and 2 years had the highest rate of ARIs at 0.56 per child-month, with those >5 years having the lowest ARI rate at 0.21 per

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of Children (% of Total Enrolled)</th>
<th>Person Time Contribution in Child-Days (% of Total Possible Child-Days From Stratum)</th>
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<td>8661 (90)</td>
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<tr>
<td>1</td>
<td>56 (24)^a</td>
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<td>2</td>
<td>45 (19)^a</td>
<td>11 835 (81)</td>
</tr>
<tr>
<td>3</td>
<td>46 (20)^a</td>
<td>10 343 (81)</td>
</tr>
<tr>
<td>4</td>
<td>22 (9)^a</td>
<td>8112 (86)</td>
</tr>
<tr>
<td>5b</td>
<td>0 (0)^a</td>
<td>1443 (79)</td>
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<tr>
<td>Gender</td>
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<tr>
<td>Female</td>
<td>119 (51)</td>
<td>28 811 (84)</td>
</tr>
<tr>
<td>Male</td>
<td>115 (49)</td>
<td>27 586 (81)</td>
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<td>Child care usage</td>
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<td>18 490 (86)</td>
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<td>Formal child care only</td>
<td>114 (49)</td>
<td>26 552 (81)</td>
</tr>
<tr>
<td>Informal and formal child care</td>
<td>22 (9)</td>
<td>4969 (75)</td>
</tr>
</tbody>
</table>

^a Data are number and percentage of children this age at time of enrollment.

b No child was >5 years of age at enrollment, per eligibility criteria. Children were able to stay on the study when they turned 5 years of age, thus contributing person time to this age stratum.
child-month (Table 2). Child care attendance significantly increased the rate of ARI by 40% (Table 2).

**Specimen Return and Viral Diagnosis**

Of the 730 ARIs identified, 543 (74%) had ≥1 specimen returned. There was no virus identified in 142 ARIs (26%), 1 virus in 347 ARIs (64%), 2 in 49 ARIs (9%), and 3 in 5 ARIs (1%). The median duration of symptoms for virus-positive ARIs was 12.0 days, with a mean of 14.2 days. Details of illnesses, including duration and health care usage, by availability of specimen and virus identification, are provided (Table 3).

Human metapneumovirus was identified in 33 illnesses (Fig 2) in 7 of the 13 months of the study, with the peak month being June 2003 (Table 3). Thirteen of the ARIs (39%) involved another virus: 5 hMPV/picornavirus coinfections, 3 hMPV/adenovirus coinfections, and 1 each of hMPV/RSV, hMPV/hCoV-NL63, hMPV/picornavirus/PIV, hMPV/picornavirus/hCoV-NL63, and hMPV/picornavirus/adenovirus coinfection. Twenty-seven (82%) of the 33 hMPV-positive children were in child care. In those ARIs where hMPV was the only virus identified, there was a general practitioner (GP) visit rate of 8.7 visits per 10 illnesses.

hCoV-NL63 was identified in 18 ARIs and, like hMPV, peaked in June (Fig 2). Ten (56%) of the ARIs involved another virus: 6 hCoV-NL63/picornavirus coinfections, and 1 each of hCoV-NL63/RSV, hCoV-NL63/influenza A virus, hCoV-NL63/hMPV, and 1 hCoV-NL63/hMPV/picornavirus coinfection. Fourteen (78%) of the 18 children positive for hCoV-NL63 were in child care. In ARIs where hCoV-NL63 was identified alone, the GP visit rate was 5 visits per 10 illnesses, and 33% were followed by a subsequent illness in ≥1 household contact.

**TABLE 2**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Acute Respiratory Illness Rate, Per Child-Month (95% CI)</th>
<th>Incidence Rate Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.50 (0.42–0.60)</td>
<td>2.39 (1.21–5.35)</td>
</tr>
<tr>
<td>1</td>
<td>0.56 (0.50–0.64)</td>
<td>2.70 (1.40–5.08)</td>
</tr>
<tr>
<td>2</td>
<td>0.51 (0.44–0.59)</td>
<td>2.44 (1.25–5.42)</td>
</tr>
<tr>
<td>3</td>
<td>0.44 (0.37–0.52)</td>
<td>2.09 (1.07–4.18)</td>
</tr>
<tr>
<td>4</td>
<td>0.39 (0.32–0.48)</td>
<td>1.88 (0.95–4.26)</td>
</tr>
<tr>
<td>5</td>
<td>0.21 (0.11–0.40)</td>
<td>Reference rate</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0.49 (0.44–0.54)</td>
<td>1.03 (0.89–1.19)</td>
</tr>
<tr>
<td>Male</td>
<td>0.48 (0.43–0.53)</td>
<td>Reference rate</td>
</tr>
<tr>
<td><strong>Child care usage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No child care</td>
<td>0.38 (0.33–0.44)</td>
<td>Reference rate</td>
</tr>
<tr>
<td>Any childcare</td>
<td>0.54 (0.49–0.58)</td>
<td>1.39 (1.19–1.65)</td>
</tr>
<tr>
<td>Informal child care only</td>
<td>0.46 (0.37–0.58)</td>
<td>1.21 (0.92–1.58)</td>
</tr>
<tr>
<td>Formal child care only</td>
<td>0.55 (0.49–0.60)</td>
<td>1.43 (1.20–1.70)</td>
</tr>
<tr>
<td>Informal and formal child care</td>
<td>0.57 (0.45–0.72)</td>
<td>1.49 (1.12–1.96)</td>
</tr>
</tbody>
</table>
Picornaviruses were the most commonly identified virus group, being present in half (269) of the ARIs where a specimen was returned, followed by adenoviruses (43 [8%]), RSV (40 [7%]), PIVs (33 [6%]), hMPV (33 [6%]), influenza A virus (24 [4%]), and hCoV-NL63 (18 [3%]). There were no identifications of influenza B virus despite its presence at Victorian sentinel influenza surveillance sites during the season. Picornavirus-related illness tended to be relatively mild, appearing least likely to be associated with fever, and having the lowest rate of primary care presentation (Table 3). Influenza A virus and adenoviruses were associated with more severe illness. Influenza A illnesses were almost universally associated with fever (95%), resulted in a similar illness in ≥1 household contact in 61% of infections, and were associated with >1 primary care attendance, on average, per illness (Table 3). Adenoviruses were identified in every month of the study, and illnesses where adenovirus was the only virus identified had a mean duration of 18.6 days. Three quarters were associated with fever and were associated with the highest rate of GP presentation, at ~13 for every 10 illnesses (Table 3). Adenovirus infections had the highest percentage of identifications with another virus present (60%). Codetections resulted in a longer median duration of illness but did not seem to impact the likelihood of fever or the rate of primary care attendances (Table 3).

**Health Care Attendances**

In the 528 ARIs with an impact diary returned, no general practice visit was recorded for 55% (292 of 528). There were 29 hospital presentations during ARIs (incidence rate: 23 presentations per 100 child years), with 5 resulting in admission (Table 3). All of the admissions resulted from a febrile respiratory illness, including 2 episodes of pneumonia; 4 had specimens collected (Table 3) with 1 RSV infection (4-year-old boy: pneumonia, admission duration 3 days), 1 influenza A virus infection (6-month-old girl: fever, dehydration, 2 days), 1 picornavirus infection (1-year-old girl: fever, wheeze, rash illness, 2 days), and 1 adenovirus/picornavirus coinfection (1-year-old girl: pneumonia, 3 days). The admission without a specimen collected (1-year-old boy) was for fever, wheeze, cough, and shortness of breath and had an admission duration of 2 days.

**DISCUSSION**

Our study adds to existing knowledge about respiratory viral infections in childhood in the following ways: it is the first to quantify the relative roles of hMPV and
hCoV-NL63 in a community sample of healthy children; it provides community-based data on common respiratory viral pathogens in children under the age of 5 years using molecular techniques; it provides initial comparative impact data for the range of respiratory viruses tested for; and it demonstrates a safe, effective, sensitive, and efficient method for the conduct of community-based studies using nucleic acid testing methods.

The frequency of ARIs, using our sensitive definition, approached 1 per child-month during the winter peak, and overall a virus could be detected by PCR testing in 74% of illnesses with a specimen available. During 2003 in Melbourne, hMPV and hCoV-NL63 circulated in the community among preschool children with other respiratory viruses. The association between child care attendance and childhood ARI has been well documented and was confirmed by this study, with the 2 viruses most strongly linked being hMPV and hCoV-NL63. Infections with hMPV resulted in relatively high rate of GP attendance at 8.7 visits per 10 illnesses, supporting findings from an Italian study, which suggested that hMPV illness had a similar impact on resource use as influenza. Comparative data for other respiratory viruses reinforce the predominant role of picornaviruses in community-managed ARIs, most likely to be rhinoviruses. Although individual illnesses caused by picornaviruses resulted in relatively milder illness, as a group they were responsible for the highest absolute number of general practice and emergency department presentations of any virus group. Although conducted during a season with higher-than-normal influenza activity, influenza A was associated with fewer but more severe illnesses (24 [4.4%]) than RSV, having 95% of infections associated with fever, the highest figure for subsequent illness in ≥1 household contact (61%), and, on average, ≥1 primary physician visit per illness.

![Figure 2](image.png)

**Figure 2**
Number of ARIs with individual virus identification according to month and virus type: Respiratory Virus Study, 2003/2004.
As with all observational studies, there are some limitations that must be considered with these data. For some viruses, the data in the impact table are generated from small numbers of infections, and point estimates should be interpreted with caution. It is reassuring that, for individual viruses, the variety of impact data, such as the presence of fever, GP visits, and likelihood of subsequent illness in a household contact, are all in agreement. Parents received details of PCR testing, including negative results, by mail as they became available, and receipt of this information may have introduced an information bias. Knowledge of the viral cause of an illness may have altered the way parents reported resource use, particularly for influenza, which is more prominent in the media. One factor would suggest that the impact of such a bias, if it occurred, is likely to be minimal: ARIs where no virus was identified seemed no less severe than other illnesses where viruses were identified.

Other recent studies have used molecular methods for virus identification in specimens from community-based infants. An English home-visit study, which followed 88 infants with ≥1 atopic, asthmatic parent during their first winter, tested for respiratory viruses, some bacterial pathogens, hCoV-OC43, and hCoV-229E, but not hMPV or hCoV-NL63, and identified a respiratory pathogen in 103 (83%) of 123 episodes. A similarly designed Western Australian study followed ARIs in 263 infants (with ≥1 parent having a diagnosis of asthma, hay fever, or eczema) during their first year (including testing for hMPV, hCoV-OC43, and hCoV-229E) and identified a virus in 69% of illnesses. This study had an unexplained very low rate of hMPV detection, being present in only 1.8% of specimens collected during illnesses. Different overall detection rates in our study and the English and Western Australian studies may be because of a number of factors, including seasonality, the relative presence of the different pathogens, the different ages and inclusion criteria for the studies, different methods for virus identification, and the nature of specimen collection (nose-throat swab versus nasal lavage or nasopharyngeal aspirate). It is likely that the proportion of all specimens from such studies with a virus identified will increase with the addition of coronavirus HKU1 and a newly identified parvovirus, human bocavirus, to testing panels.

Coronaviruses hCoV-OC43 and hCoV-229E were not tested for in our study and may account for some of the virus-negative specimens we received. In the English and Western Australian studies, these viruses were identified in 9.0% and 5.5% (including from 4.4% of control subjects) of specimens, respectively. In Victorian hospital and influenza sentinel surveillance specimens, these 2 coronaviruses (largely hCoV-OC43) were a reasonably common cause of influenza-like illness in children. They were found in 6% of influenza surveillance specimens and 12% of hospital specimens overall and peaked in August 2003, possibly accounting for the increase in virus-negative specimens that we found in August and September (Fig 1).

Our reliance on data from previous decades is most likely because of the cost of undertaking a large, community-based study: it has been suggested that this approach of studying individuals for the presence of specific viruses, as opposed to hospital-based retrospective studies, is now prohibitively expensive, presumably because of the time commitment by trained staff and travel time and costs. From a pilot study, we knew parents could recognize and document ARIs of interest, and reports in the literature suggested that parents could be trained to collect an adequate respiratory specimen. Previous community-based work on respiratory viral infections has most commonly required research staff to collect a specimen. Direct contact between subject and study staff requires additional expense but allows for the collection of clinical information. The future benefit of these data, over and above virus identification and resource consumption, is not clear. Home visits may alter health-seeking behavior and medication use, invalidating impact data.

CONCLUSIONS

There is a clear and unmet need for accurate and timely information about the community-based epidemiology of previously known and recently identified respiratory viruses in all age groups. Hospital-based studies and even community studies based around primary care have the potential to miss most illnesses: primary care physicians were consulted in only 45% of ARIs in this study. Options are currently available for the prevention and treatment of influenza with possibilities for other viruses, including vaccines and intranasal short interfering RNAs. We have shown that it is feasible to conduct large, community-based studies with personal or parent specimen collection using sensitive molecular techniques for diagnosis. Future studies should preferably be conducted in all ages at centers with concurrent hospital-based surveillance of respiratory viruses for comparison. Specimens should be tested for known viruses and stored for retesting when new pathogens are recognized. This approach also provides an efficient way of conducting vaccine or treatment efficacy studies requiring hundreds or thousands of participants.

ACKNOWLEDGMENTS

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We thank the research staff who assisted with the conduct of this study: Janet Briggs, Clare Brophy, Jim Buttery, Samantha Colquhoun, Dale Cooper, Susan Gabriel, Genevieve Hamilton, Tara Harris, Marita Keford, Betty Lim, Ethna Macken, Bernadette McCudden, Liz McGrath, Sally Mizrahi, Jane Nelson, Kerry-Ann O’Grady, Jacinta O’Sullivan, Jan Renehan, Jane Ryrie, Pam Sinclair, Amanda Tehan, and Helen Worland. We also thank laboratory staff Seweryn Białasiewicz and Katherine Arden (Queensland), who tested specimens, and Kris Jamsen and Suzanna Vidmar, who assisted with the Stata programming. This study would not have been possible without the generous support of the Maternal and Child Health Nurses from the greater Melbourne area and the children and families who volunteered, kept study documents, and collected specimens diligently.

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Community Epidemiology of Human Metapneumovirus, Human Coronavirus NL63, and Other Respiratory Viruses in Healthy Preschool-Aged Children Using Parent-Collected Specimens


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DOI: 10.1542/peds.2006-3703
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