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Parent-collected respiratory specimens—A novel method for respiratory virus and vaccine efficacy research

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Summary Population-based respiratory research and vaccine efficacy studies have previously required clinic or home visits when a subject had an acute respiratory illness. This method may mean parents are unwilling to enrol their child or report an illness of interest. We conducted a community-based cohort study into respiratory illnesses in 234 pre-school aged children using parent-collected specimens. Between January 2003 and January 2004 there were 563 specimens collected from 730 identified illnesses and these were tested using a panel of respiratory virus polymerase chain reaction (PCR) assays; 409 (73%) were positive for any virus. Specimens were not more likely to be positive when collected by a healthcare worker parent, when they included a throat swab, or when a very good collection technique was reported. A delay from illness onset to specimen collection of up to 5 days did not appear to impact on sensitivity of virus identification, but a delay of six or more days with minor delays in testing saw positivity fall. Combined with daily symptom diary completion and PCR testing, parent-collected specimens are an efficient and acceptable method for the conduct of future vaccine efficacy studies and other community-based respiratory virus research.

Introduction

The advent of sophisticated molecular techniques for the routine identification of known respiratory viruses and the discovery of new pathogens has changed our understanding of these organisms and their role in human disease. Increased understanding of the burden caused by these infections has been mirrored by a parallel interest in preventative strategies that might be applied at a population level. This particularly applies to candidate vaccines and therapeutic interventions that may limit duration of symptoms and transmission.

Recent efficacy studies of influenza vaccines have relied on the traditional model of clinic or home visits, for clinical examination and data and specimen collection, when a child had an illness of interest [1–3]. Such an approach is

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cumbersome and expensive and, with an increasing number of households in developed economies having two parents working, may prove excessively onerous on families with young children. This could result in either an unrepresentative study population, incomplete reporting of significant illness, or, at the very least, logistical challenges for the recruitment and retention of study cohorts.

We suggest here a novel method that may be useful in the future conduct of respiratory virus vaccine or therapeutic efficacy studies based on daily symptom surveillance in a study child and parent-collected specimens for polymerase chain reaction (PCR) testing for the pathogen of interest. We focus particular attention on features of this method that might impact on successful specimen collection and a positive test result.

Methods

The respiratory virus study

We conducted a community-based cohort study — the respiratory virus study (ReVS) — over a 12-month period from January 2003 and concluding data collection at the end of January 2004 [4,5]. The Human Research and Ethics Committee at the Royal Children's Hospital, Melbourne, approved the study, and written informed consent for involvement was obtained at a home visit conducted by a research assistant.

Details of the methods used in the study have been provided in other papers [4,5]. We were assisted by Maternal and Child Health Nurses at 26 local councils around Melbourne to recruit subjects for the study. We also approached day care centres and used bulletin boards and staff email lists at the Royal Children's and Royal Women's Hospitals in Melbourne. Given this use of hospitals, we classified parents as healthcare workers, who may have been expected to have a better specimen collection technique, or as non-healthcare workers. Healthcare workers included doctors, nurses, ambulance paramedics, scientists in medical research or health-related service delivery, or allied health professionals (such as occupational, speech, or physical therapists).

Daily symptom diary

Parents completed a daily tick-box symptom diary. Symptoms were classified as being Category A: fever, wheezing, shortness of breath, pulmonary congestion (moist cough), pneumonia, ear infection; or Category B: runny nose (nasal congestion), sore throat, cough, muscle aches, chills, headache, irritability, decreased activity (lethargy/weakness), vomiting. A double-sided monthly A4 diary card was provided containing a daily symptom grid. Parents were provided with pre-stamped envelopes, and encouraged to return this diary, with any other study paperwork, at the end of each month.

We based acute respiratory illness (ARI) identification on a sensitive definition used for influenza-like illness (ILI) in a vaccine efficacy study [6] and that we used in a pilot study [7,8]. An ARI of interest, warranting burden diary completion and nose—throat swab (NTS) collection, had to have at least one Category A symptom or at least two Category B symptoms present on the same day. A new ARI could not commence without there being at least three preceding symptom-free days.

Specimen collection

A research assistant provided training in the process of collecting an NTS at the enrolment visit. All research assistants enrolling subjects were given standardised instructions and training in this process. Parents were given a practical demonstration in the collection of an NTS specimen, and were left with simple written instructions, including a diagram, as a guide. The offer of graduated support at the time of NTS collection was also made: beginning initially with further instruction and support over the telephone, stepping up to a research assistant visiting the home to supervise parent collection, and failing that, having the research assistant visit to collect the specimen. During the course of the study, telephone support was only occasionally provided, usually when a parent was collecting the first specimen after an extended period since the enrolment visit. No parent required a home visit to assist with specimen collection.

Households were left with two wooden-shaft sterile cotton-tipped swabs for specimen collection (Copan red capped swab in labelled tube, 150C), a polystyrene cooler box (esky), an ice-brick, and a tube of viral transport media (VTM)—produced in-house using a standard recipe at the Victorian Infectious Diseases Reference Laboratory (VIDRL). Both the VTM and ice-brick were stored in the household freezer. Collecting the nasal swab involved rubbing a single swab against the internal anterior walls of both nostrils. We anticipated that there may be situations where parents might be reluctant to collect a throat swab or have difficulty doing so. At the enrolment visit we asked that parents make a reasonable attempt to collect a throat swab for every ARI. We asked the throat swab be collected after the nasal swab as we perceived this to be the more difficult specimen to collect and that failure to secure an adequate throat swab specimen as the first specimen might result in the subject becoming distressed and refusing further intervention. We acknowledged that it would not always be possible to collect an NTS and let parents know we would accept a nose-only swab, and provided a check box on the request slip to inform us of specimen type, consisting of a combined nose—throat swab or a nose-only swab. Also on the request slip we asked parents to subjectively rate their collection technique as very good, good, or poor. Both the nose and throat swab were pooled in the single VTM tube. The VTM and request form were put in a sealed plastic biohazard bag and placed with an ice-brick in the esky for collection. The esky was couriered initially to VIDRL for virus testing and then transported at study end to the Queensland Paediatric Infectious Diseases (Qpid) Laboratory where they were further tested for a number of respiratory viruses (Table 3) as described previously [4]. Some parents provided more than one specimen for an ARI. All specimens tested and reported in this manuscript were collected during an illness that met the ARI definition; there were no asymptomatic control specimens collected from study subjects.

Table 1 Frequency of symptoms in study children during the respiratory virus study

Symptom	Symptom category	Days present	(%)
No symptom		43,677	(77.4)
Any symptom		12,720	(22.6)
Fever	A	849	(1.5)
Wheeze	A	248	(0.4)
Shortness of breath	A	132	(0.2)
Pulmonary congestion (moist cough)	A	905	(1.6)
Pneumonia	A	20	(0.0)
Ear infection	A	428	(0.8)
Runny nose (nasal congestion)	B	9,024	(16.0)
Sore throat	B	568	(1.0)
Cough	B	5,027	(8.9)
Muscle aches	B	46	(0.1)
Chills	B	119	(0.2)
Headache	B	72	(0.1)
Irritability	B	1,671	(3.0)
Decreased activity (lethargy, weakness)	B	918	(1.6)
Vomiting	B	354	(0.6)

Household contacts

With the onset of illness in a study child we asked for surveillance to commence to identify similar illnesses in household contacts occurring within 7 days of symptoms in the study child. When such an illness occurred, we asked for completion of a symptom diary, burden diary, and collection of an NTS from the contact. These contact NTS specimens have not yet been tested for viruses.

Study conclusion questionnaire

At completion of ReVS we asked families their opinion about aspects of the study using a short, mailed questionnaire. ReVS followed children for a single year; we asked parents what their likely response would have been if we had asked them to continue in the study for another year, as might be the case in larger, prolonged community-based study. We asked parents to nominate the single most difficult study related task from a list, including tasks involving household contacts. Our research group conducts community-based vaccine studies, and we were also interested in the likelihood parents would include their child in a hypothetical experimental respiratory virus vaccine study, conducted for 5 months over the winter season. We described this study as including daily symptom diary completion and collecting an NTS when the child had an ARI.

Statistical analysis

Two-sided, two-sample tests of proportion were performed to compare virus positivity and a χ^2-test for trend was performed when comparing proportions of specimens positive for any virus with reported quality of collection. We used a level of $p < 0.05$ for significance and analyses were performed using Intercooled Stata 9.2 for Windows (StataCorp., TX, USA).

Results

Subjects were enrolled between 17 January 2003 and 05 November 2003. The last day of data collection for all subjects was 31 January 2004. Two hundred and thirty-four children were enrolled with 229 returning at least one of the monthly symptom diaries, providing 56,397 child-days of data. This represented 82.5% of 68,400 possible child-days of data had all diaries been returned. There was at least one symptom present on 12,720 (22.6%) study days, with Category B more common than Category A symptoms (Table 1). The most frequent symptom was runny nose/nasal congestion, present on 9024 days (16.0%). Symptoms were most commonly present in June and August 2003 being found on 32% of child-days both months, and least common during January 2004, present only on 8% of child-days.

Of all study households, 229 were dual parent families and 5 were single parent families. At the enrolment interview, 26% (60/234) of study mothers and 9% (20/229) of study fathers were classified as healthcare workers. There was at least one healthcare worker in 68 (29%) households.

Of the 730 ARIs identified, no specimen was returned for 187 (25.6%) and at least one specimen was returned for 543 (74.4%): 524 ARIs with one specimen, 18 with two specimens, and one with three specimens, bringing the total number of evaluable specimens to 563. There were 409 (73%) specimens that were positive for at least one virus: 154 (27%) had no virus detected; 354 (63%) had one virus detected; 50 (9%) had two viruses detected; and 5 (1%) had three viruses detected.

We explored whether specimens were positive for any virus by a number of categories: if the collecting parent was a healthcare worker, inclusion of a throat swab, and collector reported quality of the collection method (Table 2). There was no apparent difference in the likelihood of identifying any virus by healthcare worker parent or by the collector's impression of collection quality. A throat swab was included in 70% of specimens (394/563), but its presence did not improve the likelihood of a positive result (Table 2).

Table 2 Swab positivity for any virus by collector status, specimen type, and quality

	Swab positivity	*p*-Value
Collector status		
Healthcare worker	138/185 (75%)	
Non-healthcare worker	271/378 (72%)	0.468
Specimen type		
Nose-only swab	131/169 (78%)	
Nose—throat swab	278/394 (71%)	0.090
Quality[a]		
Very good	153/202 (76%)	
Good	216/305 (71%)	0.223[b]
Poor	40/56 (71%)	0.511[b]

[a] χ^2-test for trend, p = 0.29.
[b] Compared to very good stratum.

We examined whether a throat swab made any difference to positivity for individual virus types (Table 3). For all RNA viruses the likelihood of positivity was very similar for both specimen types, other than parainfluenza viruses which were more commonly identified in a nose-only swab ($p = 0.046$). A higher proportion of specimens that included a throat swab identified an adenovirus, 9% versus 5%.

We calculated the delay between ARI onset and specimen collection, and specimen collection and testing, to see if they had any impact on swab positivity (Table 4). A short delay between onset and collection appeared to have little impact on positivity, with all specimens collected within 5 days of onset having a minimum positivity rate of 71% regardless of the delay in testing, and an overall positivity rate of 74%. The rate of positivity appeared to fall for specimens collected following a delay from onset to collection of six or more days when combined with a delay in testing of two (63% positive) or more days (61%).

There were 205 household contact illnesses identified following 175 ARIs in a study child: 148 with a single subsequent illness, 24 with two illnesses, and three with three illnesses. This resulted in the collection of 184 specimens from household contacts, with 74 (40%) of these being self-collected. These specimens are currently stored in a minus 70 °C freezer waiting complete respiratory virus PCR testing.

We received completed study conclusion questionnaires from 183 (78%) families. Most of these families (87%) reported that they would have been willing to continue with the study for another year. The most difficult study procedure was reported to be collecting a throat swab from the study child (58%), followed by completing the burden diary for the study child (21%), keeping the daily symptom diary (11%), collecting a nose swab (3%), taking swabs from household contacts (2%), and completing a burden diary for the household contacts (1%). Four percent of participants found no task difficult. When parents were asked whether they would enrol their child into a hypothetical vaccine study using similar methods to ReVS (daily symptom diary and NTS collection): 35% said yes without qualification; 45% said they would consider it, but would need to hear more about the study before deciding; the remaining 20% said no, either because they were not happy for their child to receive an experimental vaccine (13%), were not happy to collect daily symptoms and specimens (3%), or were not happy to have an experimental vaccine, nor collect daily symptoms and specimens (4%).

Discussion

Findings from our study reinforce the high prevalence and burden of respiratory symptoms, particularly during winter months, in healthy pre-school aged children, and the benefits there might be for improved prevention and control of these [4,5]. The most common symptoms were present on approximately one-third of child-days in peak months.

The positivity rate of parent-collected swabs was not affected by whether the collector worked in a health-related field or the collector's perception of collection quality. Interestingly, including a throat swab did not increase the overall virus positivity. Ours was not a simultaneous, head-to-head comparison of nose-only versus nose—throat swabs, but in this setting, nose-only specimens had a higher rate of positivity (78% vs. 71%), although this difference was not significant. This finding may have been confounded by illness severity and virus shedding. A child with more severe illness may have been shedding more virus at the time of specimen collection, and in these children parents may have been less likely to attempt collection of the additionally invasive throat specimen. The only viral

Table 3 Individual virus positivity by specimen type

Virus identified	Specimen type		*p*-Value
	Nose-only swab	Nose—throat swab	
Picornaviruses	83/169 (49%)	191/394 (48%)	0.890
Adenoviruses	9/169 (5%)	36/394 (9%)	0.126
PIVs	15/169 (9%)	18/394 (5%)	0.046
RSV	15/196 (9%)	26/394 (7%)	0.341
Influenza A	8/169 (5%)	16/394 (4%)	0.717
hMPV	9/169 (5%)	25/394 (6%)	0.642
hCoV-NL63	5/169 (3%)	14/394 (4%)	0.720
Any virus[a]	131/169 (78%)	278/394 (71%)	0.090

PIVs: parainfluenza viruses; RSV: respiratory syncytial virus; hMPV: human metapneumovirus; hCoV-NL63: human coronavirus NL63.
[a] Total numerator does not equal sum of row numerators due to the co-identification of viruses in swabs.

Table 4 Swab positivity for any virus by delay from onset to collection, and collection to testing

Delay from onset of ARI to specimen collection (days)	Delay from specimen collection to test (days)			
	≤1	2	≥3	Total
≤1	41/58 (71%)	40/53 (75%)	48/61 (79%)	129/172 (75%)
2—3	45/60 (75%)	47/63 (75%)	39/57 (68%)	131/180 (73%)
4—5	25/34 (74%)	15/22 (68%)	31/40 (78%)	71/96 (74%)
≥6	35/45 (78%)	20/32 (63%)	23/38 (61%)	78/115 (68%)
Total	146/197 (74%)	122/170 (72%)	141/196 (72%)	409/563 (73%)

group where throat swabs appeared to improve detection was the adenoviruses, where a throat swab may have been more likely to capture adenoviral persistence in the oropharynx rather than acute infection, particularly in the age group under study [9]. A recent study showed that detecting adenoviral persistence 1—12 weeks after initial positivity was not possible using nose swabs alone in children 6 months to 4 years of age [10].

In our study, a delay from illness onset to specimen collection of up to 5 days did not appear to lower positivity using our method of couriering specimens from a subject's home to a central laboratory for PCR testing. The delay from collection to testing is more difficult to interpret, but it appears that six or more days delay from onset to collection combined with even minor delays in testing are not optimal for virus identification.

In the past, using antigen-based techniques for respiratory syncytial virus diagnosis, nasal, throat, and nasopharyngeal swabs have not performed as well as nasopharyngeal aspirates (NPAs) or nasal washes, with a reduction in sensitivity of up to one-third [11]. In our study, the use of PCR and collection of specimens in the early stages of illness may have minimised differences caused by site of collection, with nose-only swabs performing as well as throat swabs for virus positivity. Our rate for detection of any virus in all specimens compares favourably with other recent community-based studies using molecular methods [12,13], and these values are similar to hospital-based findings using mainly NPAs and a comprehensive panel of respiratory virus PCR assays [14]. Ideally, future research should attempt a direct comparison of NPAs to parent-collected specimens to quantify any loss of sensitivity using this method. A recent report has shown increased return of cells using flocked swabs to collect a specimen from either the nasopharynx or the nose; but subjects reported a non-statistically significant higher pain score for use of a flocked nasopharyngeal swab compared with a rayon swab (100 mm visual analog scale for pain: 61.5 vs. 43.8 mm, $p = 0.06$) [15]. Flocked swabs were not available when we commenced this study, however, we do not believe parents would be comfortable in the collection of the more invasive and potentially more uncomfortable nasopharyngeal specimen either using a flocked swab or a standard swab at home in a study subject. The use of molecular methods for virus detection may also make any improvement in sensitivity for virus detection, using a flocked swab over an NTS, from the nasopharynx over an NTS or a nose-only swab, marginal.

This is the first large-scale implementation of a community-based study reliant on parents collecting respiratory specimens. Parents were generally positively disposed to the study, and it is of interest that a high proportion of all study families reported they would have been happy to continue for another year. The most difficult part of the study was reported as being the collection of a throat specimen from an ill study child, but, based on the swab positivity results for all but adenoviruses, it may not be required—a well collected nose swab may suffice. Use of this method for experimental studies should be considered, and it is of interest that 80% of questionnaire respondents would have been happy to consider involvement in a hypothetical respiratory virus vaccine study using the same procedures as ReVS. Modifications to the protocol could make the current method simpler and more efficient, depending on study requirements. These could include using dry swabs for specimen collection to avoid the need for viral transport media to be stored in a subject's home, transporting dry swabs through the mail to reduce the reliance on more expensive couriers, and a heightened focus on diary completion, return, and specimen collection to minimise information bias. The high proportion of self-collected specimens from household contacts also demonstrates that the method could be expanded to include all ages, which would allow for contemporary household transmission studies to be performed using modern molecular testing methods.

Based on previous streptococcal research we were confident parents could be trained to collect an adequate respiratory specimen from an ill child without ongoing supervision [16—19]. We believe parent-collected specimens are a simple and efficient means of conducting future community-based epidemiological, costing, and vaccine and therapeutics efficacy studies. Parents involved in future studies can be reassured that any concern they might have about not being a healthcare worker or having a poor collection technique is unlikely to have any impact on results. Making the effort to collect a specimen in the first instance appears to be the most important step in identifying a causative virus.

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