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Observational Research in Childhood Infectious Diseases (ORChID): a dynamic birth cohort study

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ABSTRACT

Introduction: Even in developed economies infectious diseases remain the most common cause of illness in early childhood. Our current understanding of the epidemiology of these infections is limited by reliance on data from decades ago performed using low-sensitivity laboratory methods, and recent studies reporting severe, hospital-managed disease.

Methods and analysis: The Observational Research in Childhood Infectious Diseases (ORChID) study is an ongoing study enrolling a dynamic birth cohort to document the community-based epidemiology of viral respiratory and gastrointestinal infections in early childhood. Women are recruited antenatally, and their healthy newborn is followed for the first 2 years of life. Parents keep a daily symptom diary for the study child, collect a weekly anterior nose swab and dirty nappy swab and complete a burden diary when a child meets pre-defined illness criteria. Specimens will be tested for a wide range of viruses by real-time PCR assays. Primary analyses involves calculating incidence rates for acute respiratory illness (ARI) and acute gastroenteritis (AGE) for the cohort by age and seasonality. Control material from children when they are without symptoms will allow us to determine what proportion of ARIs and AGE can be attributed to specific pathogens. Secondary analyses will assess the incidence and shedding duration of specific respiratory and gastrointestinal pathogens.

Ethics and dissemination: This study is approved by The Human Research Ethics Committees of the Children's Health Queensland Hospital and Health Service, the Royal Brisbane and Women's Hospital and The University of Queensland.

Trial registration: clinicaltrials.gov NCT01304914.

ARTICLE SUMMARY

Article focus

- Infectious diseases are a common cause of morbidity in early childhood, even in developed economies.
- A diagnostic gap exists for common respiratory and gastrointestinal syndromes, with the likelihood that as yet undiscovered pathogens are involved.
- Existing knowledge about these common illnesses relies on research conducted before the rapid developments in molecular diagnostics of recent decades or focuses on disease at the severe end of the spectrum—hospitalisations—affecting a limited number of children and discounting the burden of more common, but less severe, community-managed illness.

Key messages

- This protocol outlines a dynamic birth cohort study that will allow for a detailed description of the epidemiology of respiratory and gastrointestinal viruses during the first 2 years of life.
- The large biobank of specimens to be collated will act as a rich source of material to answer targeted research questions, including the role of virus acquisition and shedding on clinical illness and the discovery of new infectious agents.

Strengths and limitations of this study

- As study procedures, including specimen collection and return, are conducted by parents, findings will be free from Hawthorne effects due to frequent interactions with study staff.
- Systematic weekly sampling will provide a control set of specimens for the individual and the cohort, allowing quantification of virus-specific attributable risk to illness.
- Non-random recruitment and enrolment requires awareness and assessment of potential bias and confounding prior to broad-based generalisation.
- Similar studies in the past have oversampled from higher socioeconomic households, and we attempt to avoid this by using a recruitment strategy that targets pregnant women in both public and private hospital settings.

INTRODUCTION

Even in developed economies where populations have high-quality housing, sanitation, secure food and drinking water supplies, good personal hygiene standards, widespread vaccine use and access to high-quality medical care, infectious diseases remain the most

common cause of significant morbidity, and occasionally mortality, in early childhood.[1–6] Our current understanding of the epidemiology of early childhood infections is limited by reliance on community-based data from decades ago using low-sensitivity diagnostic methods,[7–9] and recent studies that primarily focus on severe, hospital-managed disease.[10 11] Much of what we know, especially with newly discovered agents, originates from hospital-based prevalence studies where more than 80% of cases are less than 2 years of age, representing the sickest 2–3% of young children seen. Experience with influenza illustrates how easily disease burden can be underestimated by extrapolating from hospital data.[12–14] Available community-based studies also have important methodological limitations, such as sampling from highly selected subject populations, lack of adequate control subjects, restricted sampling frequency and observation periods, small subject numbers and/or reporting on only a few or single agents.[13 15–18] A key methodological issue is the use of home visits by healthcare workers or the requirement for clinic visits for specimen collection. Both are likely to be an imposition on busy families, regardless of the setting, leading to biased estimates of infection events and specimen availability.[19]

The highest incidence rates of acute respiratory infections (ARI) are during the first 2 years of life where on average infants experience six to eight ARIs each year.[20] Complication rates from acute otitis media (30%) and sinusitis (8%) are also highest in this age group,[21] while 3–5% of all infants are hospitalised for viral lower respiratory tract infections, including bronchiolitis, pneumonia, croup and secondary bacterial pneumonia.[22] There is emerging evidence that infectious insults to the growing and developing lung during early childhood contribute to the pathogenesis of chronic pulmonary disorders in older children and adulthood, such as asthma,[23] chronic obstructive pulmonary disease[24 25] and bronchiectasis.[26] Young children are often household introducers, actively transmitting respiratory infections to other family members.[27] Taken together, ARIs in children result in enormous current and future costs to the healthcare system, families and society.[14]

Likewise, acute gastroenteritis (AGE) is still a major cause of childhood morbidity in high-income countries. An average of 3.2 diarrhoeal episodes per child per year has been reported globally, reaching as much as 12 episodes per child per year in some settings.[1] Viruses remain important causes of gastroenteritis in children from developed countries, even after rotavirus vaccines have been introduced. In addition to rotaviruses, noroviruses, enteric adenoviruses and astroviruses are also important enteric pathogens.[28] Nevertheless, a large diagnostic gap exists in developed countries where community-based studies of at least 1 year duration report no identifiable pathogen in approximately 40–60% of young children with AGE.[29–31] In contrast, recent work employing sensitive molecular techniques describes two healthy infants shedding a diverse range of enteric viruses almost continuously throughout the first 12–14 months of life.[32] In order to first understand and then prevent early-childhood AGE such contradictory findings need to be resolved.

As well as pathogens traditionally associated with childhood infections, there are several recently identified respiratory and gastrointestinal agents, mostly viruses, which may have an important role in the epidemiology of these illnesses. Furthermore, there are likely to be undiscovered pathogens that contribute to the remaining diagnostic gap for ARI[33] and AGE episodes.[34]

We have designed this study—Observational Research in Childhood Infectious Diseases (ORChID)—to overcome identified methodological issues and collect data on the population-based epidemiology of respiratory and gastrointestinal infections during the first 2 years of life.

METHODS AND ANALYSIS

Study design

ORChID is a 5-year prospective, community-based, longitudinal, dynamic birth cohort study of ARI and AGE episodes and respiratory and gastrointestinal pathogen detection in children during the first 2 years of life (clinicaltrials.gov: NCT01304914). Recruitment will take place over a 2-year period, the last recruited child will be followed until their second birthday, and the final year of the study will be used to complete data entry and analysis, laboratory testing of specimens and to report study findings. The progressive 2-year recruitment plan allows for seasonal and year-to-year variation in pathogen activity.[13 20]

The study is designed to allow families to be self-sufficient and avoid unnecessary contact with study staff, so as to minimise bias due to a Hawthorne effect. Parents are interviewed every 3 months to record immunisation status and changes in breast feeding and childcare attendance.

An initial visit is undertaken once a child is delivered, preferably while the mother and child are still in hospital. At this visit, consent for participation is confirmed, an initial anterior nose swab and nappy swab are collected from the study child, and parents are taught the process for collecting these specimens, nasal swabs are collected from parents, diaries and study paperwork are reviewed and arrangements are made to retrieve cord blood, if available.

The Human Research Ethics Committees of the Children's Health Queensland Hospital and Health Service, the Royal Brisbane and Women's Hospital and The University of Queensland approved the study.

Study sample

Pregnant women are approached for enrolment of their newborn infants at antenatal clinics in two hospitals: one public (Royal Women's Hospital, Brisbane) and one private (Northwest Private Hospital, Everton Park). These hospitals serve communities in northern metropolitan Brisbane, a city of more than two million people,

and every year each has approximately 6100 and 1700 deliveries, respectively. Exclusion criteria for enrolment and ongoing participation include gestational age at birth of less than 36 weeks, major congenital abnormalities, chronic heart, respiratory (excluding asthma), gastrointestinal, neurological or immunological disorders, parents unable to converse in English, living outside the Brisbane metropolitan region or planning to move from the area within the next 2 years. As a dynamic cohort study, children can leave and rejoin the cohort as required.[13]

Outcomes to be measured

Following the initial visit, the study family performs the following tasks: (1) completion of the daily symptom diary, (2) weekly collection and return by mail to the research laboratory of separate anterior nose and nappy swabs and (3) when the study child has an illness that meets specified criteria, complete an impact diary.

The daily symptom diary consists of a day-by-day tick box framework, and has been modified from an ARI daily diary used in previous studies to include diarrhoea for capturing episodes of AGE.[13 35] For an ARI, one or more category A features (fever, wheezing, shortness of breath, pulmonary congestion or moist cough or medically diagnosed otitis media and/or pneumonia) or at least two category B features (runny nose or nasal congestion, sore throat, cough, muscle aches, chills, headache, irritability, decreased activity or lethargy or weakness, or any vomiting) trigger impact diary completion. For AGE, parents record daily number of vomits and number of loose stools. Diarrhoea, defined as three or more loose stools on a given day, will trigger impact diary completion. These data will allow for a gastroenteritis severity score (modified Vesikari score) validated for ambulatory settings,[36] to be calculated. The impact diary collects information on healthcare visits, including hospitalisations, diagnostic investigations, use of antibiotics, missed childcare and parental time away from work or usual activities.[13 35]

Parents collect an anterior nose swab and nappy swab from the study child once a week using a plastic-shaft, rayon budded swab, which comes with a transport tube with a foam pad reservoir soaked with viral transport medium (Virocult MW950, Medical Wire & Equipment, Wiltshire, England). For the nose specimen, a single swab is used to sample each nostril. Both specimens are sent to the research laboratory by surface mail[37 38] where they are stored in a −80° freezer.

Nose and nappy specimens are batch tested for viruses using previously validated and reported real-time PCR assays, or PCR assays developed specifically for this study (table 1). Reverse transcription precedes PCR for RNA viruses. Specimens are spiked with a known concentration of Equine Herpes Virus before extraction to assess extraction efficiency and for the presence of PCR inhibitors. The quality of respiratory and stool specimen collection are assessed by evaluating for the presence of a marker of human genomic DNA, ERV3.[37] Appropriate positive and negative controls are included in every run.

When more than one pathogen is detected simultaneously in respiratory and stool specimens, we perform a semiquantitative analysis of individual viral nucleic acids based on the cycle threshold (Ct) value.[55 56] To further differentiate between human rhinovirus (HRV) types a nested PCR-based typing system encompassing partial

Table 1 Specimen type, pathogens and assay details, ORChID study, Brisbane, Queensland, Australia

Nose swab Organism	References	Nappy swab Organism	References
Rhinoviruses	Lu *et al*[39]	Rhinoviruses	As for respiratory viruses
	Arden *et al*[40]	Coronaviruses	
HRSV-A and HRSV-B	Lambert *et al*[41]	Bocavirus	
Influenza A and influenza B			
Adenoviruses		Enteroviruses	Maunula *et al*[42]
Parainfluenza viruses I, II, III		Saffold viruses	Wang *et al*[43]
HMPV		Rotaviruses	Freeman *et al*[44]
			Jothikumar *et al*[45]
			Pang *et al*[46]
Bocavirus	Tozer *et al*[47]	Noroviruses	Kageyama *et al*[48]
hPyV-WU	O'Grady *et al*[37]	Adenoviruses	To be developed for this study
hPyV-KI	Bialasiewicz *et al*[49]	Saporovirus	
Coronavirus OC43	Gunson *et al*[50]	Astroviruses	
Coronavirus 229E		Parechoviruses	
Coronavirus NL63		Cosavirus	
Coronavirus HKU1	Dare *et al*[51]	Klassevirus	
Bordetella pertussis	Probert *et al*[52]		
Mycoplasma pneumonia	Loens *et al*[53]		
Chlamydiales species	Hirama *et al*[54]		

HRSV, human respiratory syncytial virus; HMPV, human metapneumovirus; hPyV, human polyomavirus.

sequence of the 5′ untranslated region (5′UTR) is being used. It accurately groups existing and newly identified HRV and human enterovirus strains into clades, which reflect current species assignments. Sequencing the 540 bp internal amplicon provides a rapid method to assign new sequences to a genotype.[57 58]

Nasal and nappy samples from children during illness periods where a known agent cannot be identified will be used for further investigations for the presence of as-yet-unidentified organisms. Pathogen-negative specimens will be prioritised on the basis of disease severity (hospital admission, fever). The methods employed for new pathogen detection will include pan-viral DNA microarrays[59] and 'next generation' high-throughput sequencing.[60 61]

The endpoints of the first phase of this study are (1) documentation of clinical and epidemiological data from children with a variety of syndromic illnesses which, along with exhaustive diagnostic testing using sensitive molecular methods, will allow for detailed identification and characterisation of pathogen–disease associations, (2) establishing a well-characterised collection of stored specimens with pathogen-testing results, which will be invaluable for new virus discovery and (3) determining the pathogenesis of newly identified agents from the human respiratory and gastrointestinal tracts and better defining the role of known pathogens, such as HRVs, detected in asymptomatic children.

Sample size and data analysis plan

As respiratory syncytial virus (RSV) is the most widely recognised respiratory agent associated with severe disease in this age group, we chose it to use for sample size calculations. To calculate the proportion of pathogens detected that are RSV to within ±2.5%, we assumed the current proportion to be approximately 12.5%, that conservatively each participant will have an average of eight episodes of ARI over the course of the study,[13 20 62] the intraclass correlation coefficient within individuals is 0.07 (calculated from Lambert *et al*[13]) and that 90% of subjects will remain enrolled at study completion (previous large cohort studies conducted by the investigators had retention rates of 97–98%).[13 63] Consequently, with α=0.05 and power of 80%, we were required to enrol 138 infants. Similarly, if each subject returns 80% of their weekly nasal swabs,[13] we will be able to estimate the proportion of all swabs positive for RSV to within ±0.5%.

Primary analyses will concern the calculation of incidence rates for ARI and AGE in study children for the cohort as a whole, and by age and seasonality. The collection of control material from children when they are without symptoms will allow us to determine what proportion of ARIs and AGE can be attributed to the presence of specific pathogens. Secondary analyses will assess the incidence and shedding duration of respiratory and gastrointestinal pathogens. Analyses will be conducted with mixed effects models with random intercepts and slope (time effect) for each participant. This method controls for the non-independence in outcomes from the same participant at different times and allows for heterogeneity between participants.

DISCUSSION

Infections are responsible for a significant burden during the early childhood years. We have presented here the study protocol and data analysis plans for an ambitious observational study: ORChID. The study has been designed to overcome identified weaknesses in previous research, and to use modern molecular techniques to identify infecting agents. The real value of this study is in the establishment of a biobank of thousands of respiratory and gastrointestinal specimens linked with daily clinical data. As well as the testing we outline here, we will in future be able to use the biobank for rapid and detailed assessments of the clinical significance of newly identified pathogens using highly sensitive molecular testing.

Strengths of the study include that it is community-based and uses parent-collected specimens returned to the diagnostic laboratory by surface mail for highly sensitive and specific real-time PCR testing for a comprehensive range of viruses. Systematic weekly sampling provides a control set of specimens, from both the same study child and all study children, during asymptomatic periods that can act as a control for specimens collected during periods of illness. This will allow us to quantify the attributable risk of pathogen detection to illness.

It would not be logistically or economically possible to conduct a study on this scale without using parent-collected specimens that are returned to the laboratory by surface mail. We and others have demonstrated that collection of respiratory specimens by non-healthcare workers is feasible[19 41 64–69] and, when combined with sensitive molecular diagnostics, does not result in any overall significant reduction in sensitivity for virus detection.[19 41 65 68] In a small randomised controlled trial, parent collection was no worse than a home visit by a healthcare worker for both having a specimen collected during an illness and having a virus identified when specimens were tested; the trend for both measures was improved collection and identification with parent collection.[19] Further, the swabs we are using have been shown to be similarly sensitive for the detection of influenza nucleic acid when compared with flocked swabs combined with a universal transport media.[70] We have found that return of specimens using surface mail did not result in reduced sensitivity when compared to immediate and maintained freezing of specimens, even over long distances.[37] Mailing specimens has also been shown to be an efficient means of community-based research in other settings.[66 67]

There remain diagnostic gaps in the identification of the causative agent in ARI and AGE.[33 34] We have been involved in the discovery and fuller description of newly

identified pathogens from the human respiratory and gastrointestinal tract.[40 47 49 60 71–86] The ORChID study will allow us to link specimens negative for known pathogens with symptoms and illness impact data, enabling us to prioritise specimens for new pathogen discovery. As well as the real possibility of discovering new agents, the epidemiology of known viruses, including their interactions,[87] will be analysed.

Like most observational studies, recruitment is non-random, requiring assessment of potential biases and confounders prior to considering the internal and external validity of any findings. Where reported, oversampling of families from higher socioeconomic households appears common in research similar to this study,[14 63 88] although differences in incidence and cost of illnesses mean that any impact on burden assessment is likely to be minimal and biased towards the null. We have attempted to avoid this selection bias by using a large, public hospital as one of our two recruitment sites. The role household socioeconomic status plays on incidence and disease burden will be assessed in the study analysis.

Recruitment for the study started on 12 August 2010 with the first study baby born on 15 September 2010. Recruitment will continue for the 2 years planned and is expected to conclude in December 2012. To date (31 August 2012), there have been 163 pregnant women recruited and 148 children enrolled. We are currently receiving over 350 nasal and nappy swabs a month, for a total return of more than 6000 of each specimen type. Batch testing of the nasal swabs for respiratory viruses has started with 5200 nasal swabs tested for 17 respiratory viruses to date, with laboratory staff blind to symptom presence in study subjects.

CONCLUSION

The ORChID study is well underway having started recruitment of pregnant women and their newborns for 2 years of intensive respiratory and gastrointestinal specimen collection linked with daily clinical data in 2010. Over 12 000 specimens have been returned from 148 enrolled infants, with blinded real-time PCR started for respiratory viruses. The study will provide unique insights on the acquisition of viruses, duration of shedding and the relationship of these to symptomatic illness. As well as this, specimens will be available for targeted discovery of new pathogens, and will provide a rich source of material to define the epidemiology of new agents when described by others.

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Contributors KG is the study principal investigator. SBL, RW, DW, TPS, MDN and KG developed the successful grant application which supports the study. ALC and FAM are study nurses responsible for subject recruitment, enrollment and cohort maintenance. DMW, SB and IMM provide technical expertise for diagnostic methods. SBL drafted the manuscript. All authors critically revised the manuscript for important intellectual content, contributed to and approved the final version of the manuscript for publication.

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Competing interests None.

Ethics approval The Human Research Ethics Committees of the Children's Health Queensland Hospital and Health Service, the Royal Brisbane and Women's Hospital and The University of Queensland.

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REFERENCES

1. Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull World Health Organ* 2003;81:197–204.
2. Mulholland K. Global burden of acute respiratory infections in children: implications for interventions. *Pediatr Pulmonol* 2003;36:469–74.
3. Carville KS, Lehmann D, Hall G, *et al*. Infection is the major component of the disease burden in Aboriginal and non-Aboriginal Australian children: a population-based study. *Pediatr Infect Dis J* 2007;26:210–16.
4. Ladhani S, Pebody RG, Ramsay ME, *et al*. Continuing impact of infectious diseases on childhood deaths in England and Wales, 2003–2005. *Pediatr Infect Dis J* 2010;29:310–13.
5. Depani SJ, Ladhani S, Heath PT, *et al*. The contribution of infections to neonatal deaths in England and Wales. *Pediatr Infect Dis J* 2011;30:345–7.
6. McClarren RL, Lynch B, Nyayapati N. Acute infectious diarrhea. *Prim Care* 2011;38:539–64; ix.
7. Higgins MW, Longini IM. The Tecumseh Community Health Study. *Prog Clin Biol Res* 1984;147:43–5.
8. Monto AS, Koopman JS, Longini IM, *et al*. The Tecumseh study. XII. Enteric agents in the community, 1976–1981. *J Infect Dis* 1983;148:284–91.
9. Longini IM Jr., Koopman JS, Monto AS, *et al*. Estimating household and community transmission parameters for influenza. *Am J Epidemiol* 1982;115:736–51.
10. Moore HC, de Klerk N, Richmond P, *et al*. Seasonality of respiratory viral identification varies with age and Aboriginality in metropolitan Western Australia. *Pediatr Infect Dis J* 2009;28:598–603.
11. Malek MA, Curns AT, Holman RC, *et al*. Diarrhea- and rotavirus-associated hospitalizations among children less than 5 years of age: United States, 1997 and 2000. *Pediatrics* 2006;117:1887–92.
12. Poehling KA, Edwards KM, Weinberg GA, *et al*. The underrecognized burden of influenza in young children. *N Engl J Med* 2006;355:31–40.
13. Lambert SB, Allen KM, Druce JD, *et al*. Community epidemiology of human metapneumovirus, human coronavirus NL63, and other respiratory viruses in healthy preschool-aged children using parent-collected specimens. *Pediatrics* 2007;120:e929–37.
14. Lambert SB, Allen KM, Carter RC, *et al*. The cost of community-managed viral respiratory illnesses in a cohort of healthy preschool-aged children. *Respir Res* 2008;9:11.

15. von Linstow ML, Hogh M, Hogh B. Clinical and epidemiologic characteristics of human bocavirus in Danish infants: results from a prospective birth cohort study. *Pediatr Infect Dis J* 2008;27:897–902.
16. Jackson DJ, Gangnon RE, Evans MD, *et al*. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med* 2008;178:667–72.
17. White LJ, Buttery J, Cooper B, *et al*. Rotavirus within day care centres in Oxfordshire, UK: characterization of partial immunity. *J R Soc Interface* 2008;5:1481–90.
18. Bishop RF, Bugg HC, Masendycz PJ, *et al*. Serum, fecal, and breast milk rotavirus antibodies as indices of infection in mother-infant pairs. *J Infect Dis* 1996;174(Suppl 1):S22–9.
19. van der Zalm MM, Uiterwaal CS, de Jong BM, *et al*. Viral specimen collection by parents increases response rate in population-based virus studies. *J Allergy Clin Immunol* 2006;117:955–6; author reply 56–7.
20. Monto AS. Epidemiology of viral respiratory infections. *Am J Med* 2002;112(Suppl 6A):4S–12S.
21. Revai K, Dobbs LA, Nair S, *et al*. Incidence of acute otitis media and sinusitis complicating upper respiratory tract infection: the effect of age. *Pediatrics* 2007;119:e1408–12.
22. van Woensel JB, van Aalderen WM, Kimpen JL. Viral lower respiratory tract infection in infants and young children. *BMJ* 2003;327:36–40.
23. Holt PG, Sly PD. Viral infections and atopy in asthma pathogenesis: new rationales for asthma prevention and treatment. *Nat Med* 2012;18:726–35.
24. de Marco R, Accordini S, Marcon A, *et al*. Risk factors for chronic obstructive pulmonary disease in a European cohort of young adults. *Am J Respir Crit Care Med* 2011;183:891–7.
25. Svanes C, Sunyer J, Plana E, *et al*. Early life origins of chronic obstructive pulmonary disease. *Thorax* 2010;65:14–20.
26. Chang AB, Byrnes CA, Everard ML. Diagnosing and preventing chronic suppurative lung disease (CSLD) and bronchiectasis. *Paediatr Respir Rev* 2011;12:97–103.
27. Peltola V, Waris M, Osterback R, *et al*. Rhinovirus transmission within families with children: incidence of symptomatic and asymptomatic infections. *J Infect Dis* 2008;197:382–9.
28. Higgins G, Schepetiuk S, Ratcliff R. Aetiological importance of viruses causing acute gastroenteritis in humans. *Microbiology Australia* 2012;33:49–52.
29. de Wit MA, Koopmans MP, Kortbeek LM, *et al*. Etiology of gastroenteritis in sentinel general practices in The netherlands. *Clin Infect Dis* 2001;33:280–8.
30. Iturriza-Gomara M, Elliot AJ, Dockery C, *et al*. Structured surveillance of infectious intestinal disease in pre-school children in the community: 'The Nappy Study'. *Epidemiol Infect* 2009;137:922–31.
31. Grant L, Vinje J, Parashar U, *et al*. Epidemiologic and clinical features of other enteric viruses associated with acute gastroenteritis in American Indian infants. *J Pediatr* 2012;161:110–15. e1.
32. Kapusinszky B, Minor P, Delwart E. Nearly Constant Shedding of Diverse Enteric Viruses by Two Healthy Infants. *J Clin Microbiol* 2012;50:3427–34.
33. Sloots TP, Whiley DM, Lambert SB, *et al*. Emerging respiratory agents: new viruses for old diseases? *J Clin Virol* 2008;42:233–43.
34. Vernacchio L, Vezina RM, Mitchell AA, *et al*. Diarrhea in American infants and young children in the community setting: incidence, clinical presentation and microbiology. *Pediatr Infect Dis J* 2006;25:2–7.
35. Lambert S, O'Grady KA, Gabriel S, *et al*. The cost of seasonal respiratory illnesses in Australian children: the dominance of patient and family costs and implications for vaccine use. *Commun Dis Intell* 2004;28:510–16.
36. Freedman SB, Eltorky M, Gorelick M. Evaluation of a gastroenteritis severity score for use in outpatient settings. *Pediatrics* 2010;125: e1278–85.
37. O'Grady KA, Torzillo PJ, Rockett RJ, *et al*. Successful application of a simple specimen transport method for the conduct of respiratory virus surveillance in remote Indigenous communities in Australia. *Trop Med Int Health* 2011;16:766–72.
38. Yin JK, Lahra MM, Iskander M, *et al*. Pilot study of influenza vaccine effectiveness in urban Australian children attending childcare. *J Paediatr Child Health* 2011;47:857–62.
39. Lu X, Holloway B, Dare RK, *et al*. Real-time reverse transcription-PCR assay for comprehensive detection of human rhinoviruses. *J Clin Microbiol* 2008;46:533–9.
40. Arden KE, Mackay IM. Newly identified human rhinoviruses: molecular methods heat up the cold viruses. *Rev Med Virol* 2010;20:156–76.
41. Lambert SB, Whiley DM, O'Neill NT, *et al*. Comparing nose-throat swabs and nasopharyngeal aspirates collected from children with symptoms for respiratory virus identification using real-time polymerase chain reaction. *Pediatrics* 2008;122:e615–20.
42. Maunula L, Klemola P, Kauppinen A, *et al*. Enteric viruses in a large waterborne outbreak of acute gastroenteritis in Finland. *Food Environ Virol* 2009;1:31–6.
43. Wang CYT, Greer RM, Delwart E, *et al*. A newly designed real-time RT-PCR for SAFV detects SAFV-2 and SAFV-3 in the respiratory tracts of ill children during 2011. *J Clin Virol* 2012;55:173–76.
44. Freeman MM, Kerin T, Hull J, *et al*. Enhancement of detection and quantification of rotavirus in stool using a modified real-time RT-PCR assay. *J Med Virol* 2008;80:1489–96.
45. Jothikumar N, Kang G, Hill VR. Broadly reactive TaqMan assay for real-time RT-PCR detection of rotavirus in clinical and environmental samples. *J Virol Methods* 2009;155:126–31.
46. Pang X, Cao M, Zhang M, *et al*. Increased sensitivity for various rotavirus genotypes in stool specimens by amending three mismatched nucleotides in the forward primer of a real-time RT-PCR assay. *J Virol Methods* 2011;172:85–7.
47. Tozer SJ, Lambert SB, Whiley DM, *et al*. Detection of human bocavirus in respiratory, fecal, and blood samples by real-time PCR. *J Med Virol* 2009;81:488–93.
48. Kageyama T, Kojima S, Shinohara M, *et al*. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. *J Clin Microbiol* 2003;41:1548–57.
49. Bialasiewicz S, Whiley DM, Lambert SB, *et al*. Development and evaluation of real-time PCR assays for the detection of the newly identified KI and WU polyomaviruses. *J Clin Virol* 2007;40:9–14.
50. Gunson RN, Collins TC, Carman WF. Real-time RT-PCR detection of 12 respiratory viral infections in four triplex reactions. *J Clin Virol* 2005;33:341–4.
51. Dare RK, Fry AM, Chittaganpitch M, *et al*. Human coronavirus infections in rural Thailand: a comprehensive study using real-time reverse-transcription polymerase chain reaction assays. *J Infect Dis* 2007;196:1321–8.
52. Probert WS, Ely J, Schrader K, *et al*. Identification and evaluation of new target sequences for specific detection of Bordetella pertussis by real-time PCR. *J Clin Microbiol* 2008;46:3228–31.
53. Loens K, Beck T, Ursi D, *et al*. Evaluation of different nucleic acid amplification techniques for the detection of M. pneumoniae, C. pneumoniae and Legionella spp. in respiratory specimens from patients with community-acquired pneumonia. J Microbiol Methods 2008;73:257–62.
54. Hirama T, Yamaguchi T, Miyazawa H, *et al*. Prediction of the pathogens that are the cause of pneumonia by the battlefield hypothesis. *PLoS One* 2011;6:e24474.
55. Christensen A, Nordbo SA, Krokstad S, *et al*. Human bocavirus commonly involved in multiple viral airway infections. *J Clin Virol* 2008;41:34–7.
56. Gerna G, Campanini G, Rognoni V, *et al*. Correlation of viral load as determined by real-time RT-PCR and clinical characteristics of respiratory syncytial virus lower respiratory tract infections in early infancy. *J Clin Virol* 2008;41:45–8.
57. Lee WM, Kiesner C, Pappas T, *et al*. A diverse group of previously unrecognized human rhinoviruses are common causes of respiratory illnesses in infants. *PLoS One* 2007;2:e966.
58. Mackay IM, Lambert SB, Faux CE, *et al*. Community-wide, contemporaneous circulation of a broad spectrum of human rhinoviruses in healthy Australian preschool-aged children during a 12-month period. *J Infect Dis* 2012. epub ahead of print; doi: 10. 1093/infdis/jis476
59. Wang D, Urisman A, Liu YT, *et al*. Viral discovery and sequence recovery using DNA microarrays. *PLoS Biol* 2003;1:E2.
60. Gaynor AM, Nissen MD, Whiley DM, *et al*. Identification of a novel polyomavirus from patients with acute respiratory tract infections. *PLoS Pathog* 2007;3:e64.
61. Finkbeiner SR, Li Y, Ruone S, *et al*. Identification of a novel astrovirus (astrovirus VA1) associated with an outbreak of acute gastroenteritis. *J Virol* 2009;83:10836–9.
62. Kusel MM, de Klerk N, Holt PG, *et al*. Occurrence and management of acute respiratory illnesses in early childhood. *J Paediatr Child Health* 2007;43:139–46.
63. Lambert SB, O'Grady KF, Gabriel SH, *et al*. Respiratory illness during winter: a cohort study of urban children from temperate Australia. *J Paediatr Child Health* 2005;41:125–9.
64. Elliot AJ, Powers C, Thornton A, *et al*. Monitoring the emergence of community transmission of influenza A/H1N1 2009 in England: a cross sectional opportunistic survey of self sampled telephone callers to NHS Direct. *BMJ* 2009;339:b3403.
65. Esposito S, Molteni CG, Daleno C, *et al*. Collection by trained pediatricians or parents of mid-turbinate nasal flocked

swabs for the detection of influenza viruses in childhood. *Virol J* 2010;7:85.
66. Akmatov MK, Krebs S, Preusse M, *et al*. E-mail-based symptomatic surveillance combined with self-collection of nasal swabs: a new tool for acute respiratory infection epidemiology. *Int J Infect Dis* 2011;15: e799–803.
67. van der Zalm MM, Wilbrink B, van Ewijk BE, *et al*. Highly frequent infections with human rhinovirus in healthy young children: a longitudinal cohort study. *J Clin Virol* 2011;52:317–20.
68. Ip DK, Schutten M, Fang VJ, *et al*. Validation of self-swab for virologic confirmation of influenza virus infections in a community setting. *J Infect Dis* 2012;205:631–4.
69. Lambert SB, Allen KM, Nolan TM. Parent-collected respiratory specimens—a novel method for respiratory virus and vaccine efficacy research. *Vaccine* 2008;26:1826–31.
70. Esposito S, Molteni CG, Daleno C, *et al*. Comparison of nasopharyngeal nylon flocked swabs with universal transport medium and rayon-bud swabs with a sponge reservoir of viral transport medium in the diagnosis of paediatric influenza. *J Med Microbiol* 2010;59:96–9.
71. Holtz LR, Bauer IK, Rajendran P, *et al*. Astrovirus MLB1 is not associated with diarrhea in a cohort of Indian children. *PLoS One* 2011;6:e28647.
72. Holtz LR, Finkbeiner SR, Zhao G, *et al*. Klassevirus 1, a previously undescribed member of the family Picornaviridae, is globally widespread. *Virol J* 2009;6:86.
73. Finkbeiner SR, Le BM, Holtz LR, *et al*. Detection of newly described astrovirus MLB1 in stool samples from children. *Emerg Infect Dis* 2009;15:441–4.
74. Finkbeiner SR, Holtz LR, Jiang Y, *et al*. Human stool contains a previously unrecognized diversity of novel astroviruses. *Virol J* 2009;6:161.
75. Holtz LR, Finkbeiner SR, Kirkwood CD, *et al*. Identification of a novel picornavirus related to cosaviruses in a child with acute diarrhea. *Virol J* 2008;5:159.
76. Finkbeiner SR, Kirkwood CD, Wang D. Complete genome sequence of a highly divergent astrovirus isolated from a child with acute diarrhea. *Virol J* 2008;5:117.
77. Finkbeiner SR, Allred AF, Tarr PI, *et al*. Metagenomic analysis of human diarrhea: viral detection and discovery. *PLoS Pathog* 2008;4: e1000011.
78. Bialasiewicz S, Whiley DM, Lambert SB, *et al*. Presence of the newly discovered human polyomaviruses KI and WU in Australian patients with acute respiratory tract infection. *J Clin Virol* 2008;41:63–8.
79. Kistler A, Avila PC, Rouskin S, *et al*. Pan-viral screening of respiratory tract infections in adults with and without asthma reveals unexpected human coronavirus and human rhinovirus diversity. *J Infect Dis* 2007;196:817–25.
80. Bialasiewicz S, Whiley DM, Lambert SB, *et al*. A newly reported human polyomavirus, KI virus, is present in the respiratory tract of Australian children. *J Clin Virol* 2007;40:15–18.
81. Rota PA, Oberste MS, Monroe SS, *et al*. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science* 2003;300:1394–9.
82. Arden KE, Chang AB, Lambert SB, *et al*. Newly identified respiratory viruses in children with asthma exacerbation not requiring admission to hospital. *J Med Virol* 2010;82:1458–61.
83. Arden KE, Faux CE, O'Neill NT, *et al*. Molecular characterization and distinguishing features of a novel human rhinovirus (HRV) C, HRVC-QCE, detected in children with fever, cough and wheeze during 2003. *J Clin Virol* 2010;47:219–23.
84. Bialasiewicz S, Lambert SB, Whiley DM, *et al*. Merkel cell polyomavirus DNA in respiratory specimens from children and adults. *Emerg Infect Dis* 2009;15:492–4.
85. Bialasiewicz S, Whiley DM, Lambert SB, *et al*. Detection of BK, JC, WU, or KI polyomaviruses in faecal, urine, blood, cerebrospinal fluid and respiratory samples. *J Clin Virol* 2009;45:249–54.
86. Mackay IM, Lambert SB, McErlean PK, *et al*. Prior evidence of putative novel rhinovirus species, Australia. *Emerg Infect Dis* 2008;14:1823–4; author reply 24–5.
87. Greer RM, McErlean P, Arden KE, *et al*. Do rhinoviruses reduce the probability of viral co-detection during acute respiratory tract infections? *J Clin Virol* 2009;45:10–15.
88. Kusel MM, de Klerk NH, Holt PG, *et al*. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: a birth cohort study. *Pediatr Infect Dis J* 2006;25:680–6.