Published in final edited form as:
Clin Pediatr (Phila). 2011 June ; 50(6): 513–517. doi:10.1177/0009922810394834.

Viral Etiology of Acute Febrile Respiratory Illnesses in Hospitalized Children Younger Than 24 Months

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Abstract

Background—Respiratory infections are a leading cause of pediatric hospitalizations. This study investigated whether virus–virus or virus–*Bordetella* co-infections are more frequent or more severe than previously recognized.

Methods—This is a 3-year prospective study of children younger than 24 months hospitalized with a febrile respiratory illness. Viral pathogens were detected using multiplex polymerase chain reaction (PCR), enzyme-linked immunoassays, and/or viral cultures from nasopharyngeal samples. *Bordetella* infections were detected by PCR.

Results—A total of 201 patients were enrolled. Respiratory viruses were detected in 187 (93%) patients, with 52 (28%) multipathogen infections. The most common viruses detected were respiratory syncytial virus and rhinovirus/enterovirus. There were no differences in illness severity when comparing patients infected with one pathogen and those with multipathogen infection.

Conclusion—Virus co-infection in young children hospitalized with an acute febrile respiratory infection is common but does not appear to be associated with illness severity.

Keywords

respiratory infections; multiplex PCR; respiratory viral pathogens

Introduction

Approximately 400 000 children are hospitalized in the United States each year with a clinical diagnosis of viral lower respiratory tract infection (LRTI), but historically, only half of these patients ever have a viral etiology identified as the cause of their illness.[1,2] Whereas respiratory syncytial virus (RSV) accounts for the majority of these hospitalizations,[3] human metapneumovirus (hMPV), parainfluenza viruses (PIVs), rhinoviruses, enteroviruses, adenoviruses, and influenza viruses can also result in moderate to severe infections requiring hospitalization.[1,3–7] When these viruses infect the lower respiratory tract, they most often cause bronchiolitis and/or pneumonia, but less typical findings may also develop, including periodic breathing or apnea, particularly in the very young. In infants and young children,

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Declaration of Conflicting Interests
The author(s) declared no conflicts of interest with respect to the authorship and/or publication of this article.

infection with *Bordetella pertussis* or *B parapertussis*, the bacterial causes of classic pertussis syndrome, share overlapping features with viral LRTI, as at least 2 of these virus groups (RSV and adenovirus) are known causes of pertussis syndrome.

Recent studies have shown that simultaneous infection with more than one virus pathogen occurs in 17% to 36% of children diagnosed with bronchiolitis.[2,4,8–13] RSV, influenza, rhinovirus, adenovirus, and hMPV are the most common infecting agents.[2,4,7–12] Two studies, one relatively recent, also document rates of co-infection with RSV and *B pertussis* to be approximately 8%.[14,15] However, studies that have attempted to correlate dual infection with illness severity are limited and have yielded conflicting results.[4,5,12,16]

Historically, standard cell culture has been the "gold standard" for the laboratory detection of respiratory viruses.[17] This method is technically challenging and several days are required before results are available. Moreover, several of the common etiologic agents are not easily cultured using standard techniques.[18] Rapid immunoassay antigen detection tests are routinely available for the detection of RSV and influenza, but are known to have suboptimal sensitivity.[18] In contrast, detection of viral antigens in respiratory secretions by immunofluorescence has greater sensitivity but is labor intensive and is highly dependent on the experience and skill of the observer.[19] The recent emergence of PCR based technology has substantially increased the sensitivity of viral diagnostics.[18] Such assays allow for confirmatory diagnoses of agents that do not grow in standard tissue culture, and have led to the emerging recognition that simultaneous infection with multiple viral pathogens occurs regularly in children.[1,2]

We sought to determine whether virus–virus and/or virus–*Bordetella* co-infections are more frequent or more severe than previously recognized now that sensitive polymerase chain reaction (PCR) assays are available to detect a more complete set of pathogens.

Materials and Methods

Children younger than 24 months who were hospitalized with a febrile respiratory illness at our institution between October 2007 and May 2010 were eligible for enrollment in the study if the clinical team ordered any respiratory virus diagnostic tests. Signed informed consent was obtained and an additional nasopharyngeal swab specimen collected by the research team to test for *B pertussis* and *B parapertussis*. The institutional review boards of the enrolling hospitals approved the study.

Demographic, clinical, and virologic data were collected from the hospital records, including patient age, gender, ethnicity, underlying medical conditions, symptoms on admission, physical exam findings, oxygen requirement, intensive care unit admission, and length of hospital stay. Enzyme-linked immunoassays (EIA) for RSV and influenza (Binax NOW RSV and Influenza A & B Test Kits, Binax Inc, Scarborough, ME or BD Directigen EZ RSV or EZ Flu A + B Kits, Becton Dickinson, Sparks, MD) and respiratory viral cultures were performed on nasal washes and/or swabs that were collected as part of the routine clinical evaluation as dictated by the clinical team.

Between October 2007 and September 2008, samples for routine clinical care were collected by nasal wash. If the EIA results of the nasal wash specimen were negative for RSV and influenza, general respiratory viral cultures were performed. Residual nasal wash samples were evaluated by the research team using the PCR-based xTag RVP panel (Luminex Corp, Austin, TX) to identify 16 respiratory viruses: RSV A and B; influenza A and B; parainfluenza 1, 2, 3, and 4; human metapneumovirus; rhinovirus/enterovirus; adenovirus; and coronaviruses NL63, 229E, OC43, and HKU1. The clinical virology laboratory underwent an operational change beginning October 2008. Respiratory viral cultures from

nasal wash samples were no longer performed routinely. Instead, nasopharyngeal flocked swabs (Copan Diagnostics, Corona, CA) were submitted and used for RVP testing. The RVP assay, as performed for routine patient care, allowed for detection of 11 of the above-listed respiratory viruses (the tests for PIV4 and the 4 coronaviruses were not included in the Food and Drug Administration–approved version of the RVP assay), but these results were still available to us through this research protocol.

For study purposes, any/all positive results including viral culture, EIA, or RVP were considered diagnostic. The second nasal swab obtained from each patient was processed to detect *B pertussis* and *B parapertussis* DNA by PCR as per manufacturer's protocol (Diagenode Bordetella Real Time PCR, Diagenode Inc, Sparta, NJ).

Statistical Analysis

Clinical characteristics were compared using the Student's *t* test.

Results

A total of 201 children were enrolled in the study. The median age at hospitalization was 4 months, 116 (58%) were males. In total, 197 (93%) of these children had a positive virus diagnostic test (Table 1). A total of 135 (72%) patient samples contained a single virus and 52 (28%) contained multiple viruses. Overall, the most commonly detected viruses were RSV (n = 108, 58%) and rhinovirus/enterovirus (n = 61, 33%; Tables 1 and 2). Table 2 highlights the combinations of viral etiologies documented in the 50 children with dual infections. There were only 2 instances of multipathogen infections where 3 viruses were detected, including simultaneous detection of RSV, rhinovirus/enterovirus, and PIV in one sample, and simultaneous detection of RSV, rhinovirus/enterovirus, and hMPV in the other.

The most common clinical diagnoses associated with the children enrolled in this study were bronchiolitis and pneumonia (Table 3). Five children with febrile respiratory illnesses were also diagnosed with gastroenteritis; coronaviruses were detected in samples from 4 of these patients. Two children admitted with a primary diagnosis of bronchiolitis were also found to have hepatitis (peak serum ALT of 384 U/L and 549 U/L). Dual infection was documented in both of these patients; one with coronavirus HKU1 and RSV, the other with coronavirus HKU1 and rhinovirus/enterovirus.

There were no differences between patients infected with one virus and those infected with multiple pathogens with regard to oxygen requirement, intensive care unit admission, or length of hospital stay (Table 4).

Rapid EIA testing for RSV was performed on 197 of the 201 patient samples. EIA detected RSV in 80 (41%) of the samples whereas PCR identified RSV in 104 of the same 197 samples (53%). Of the 94 viral cultures that were performed, 28 (30%) were positive, although none showed replication of more than one virus. In contrast, RVP analysis of the same 94 samples detected nucleic acid for more than one agent in 24 samples (26%).

Of the 201 children tested, one patient tested positive for *B pertussis* and one for *B parapertussis*. The patient with *B pertussis* was co-infected with RSV.

Discussion

In this work, we describe the etiologies of acute febrile respiratory illness in hospitalized children younger than 24 months using diagnostic testing to detect 16 viruses and *B pertussis* and *B parapertussis*. We detected at least one virus in 93% of enrolled children.

Historically, virus detection rates of 47 to 95% have been described.[2,8,9,11,13,20,21] Among the 14 patients in our study for whom a viral etiology could not be determined, 1 was diagnosed with *B parapertussis* infection, and the remaining 13 were diagnosed clinically with bronchiolitis and/or viral syndrome. These cases could have been because of noninfectious causes, atypical bacterial disease (*Chlamydophila pneumoniae*, *Chlamydia trachomatis*, *Mycoplasma pneumonia*), unconfirmed pneumococcal infection, a yet to be identified virus, a virus normally detected by RVP, but because of sampling or testing issues went undetected, or a virus not detected by RVP such as human bocavirus. Of note, Allander et al[13] described human bocavirus to be detected in 19% of children hospitalized with an acute wheezing episode.

Overall, 26% of our patients were simultaneously infected with multiple pathogens, a co-infection rate within the published range of 17% to 36%.[2,8–13] Six (12%) of these patients were dually infected with viruses that each require different hospital isolation procedures, specifically involving co-infections with adenovirus and influenza virus, which require droplet precautions.

There have been conflicting reports regarding multipathogen viral infections and its association with the severity of acute disease. In 2003, Greensill et al[5] showed that 70% of infants with severe RSV disease were co-infected with hMPV, and Richard et al[4] found that infants with dual infections were 2.7 times more likely to require pediatric intensive care unit admission when compared with those with a single agent infection. In contrast, Marguet et al[16] found that co-infection with RSV and rhinovirus did not result in more severe disease in hospitalized infants when compared with infection with either agent alone, and Peng et al[9] found no differences in illness severity between infections caused by single or multiple viruses. Our data support the latter conclusion. We found no differences in length of hospital stay, likelihood of intensive care unit admission, or need for supplemental oxygen among hospitalized infants when we compared those infected with one virus to those infected with more than one virus. Since hospitalization was an enrollment criterion for our study we do not address the possibility that infection with more than one virus was an independent risk factor leading to hospitalization.

Inclusion criteria for enrollment dictated that all patients in our study had a febrile respiratory illness. Nine (4%) of our patients also had prominent gastrointestinal complaints, 6 with gastroenteritis and 3 with hepatitis. Those with gastroenteritis were diagnosed with rhinovirus/enterovirus infection (n = 1), adenovirus infection (n = 1), and coronavirus infection (1 with coronavirus OC43, 3 with coronavirus NL63). Of the patients with hepatitis, 1 had adenovirus infection, the other 2 had dual infections with coronavirus HKU1 and either RSV or rhinovirus/enterovirus. Although it is unclear whether the HKU1 coronavirus caused or contributed to the hepatitis in either case, it is of interest that several animal species of coronaviruses are primarily tropic to the gut and/or liver.[22–24]

During our study period, the global H1N1 pandemic caused a substantial burden on our health care system resulting in more than 86 000 pediatric hospitalizations in the United States alone. We were therefore surprised to find only 3 patients with influenza infection in our study group. There are several possible explanations for this observation. A study from Melbourne, Australia found that the majority of influenza-like illnesses in children younger than 2 years during the 2009 H1N1 influenza pandemic was actually caused by RSV.[25] Infants infected with influenza virus often present with fever no source, lacking respiratory symptoms altogether.[26] Such patients would not have been eligible for inclusion in the present study.

Although distinguishing between respiratory infections due to a single virus and those resulting from multiple pathogens may not change individual patient management, it does change cohorting practices and hospital isolation procedures. Patients hospitalized with RSV, rhinovirus/enterovirus, hMPV, coronavirus, or PIV infections are placed on contact precautions. In contrast, patients infected with adenovirus, influenza, or *B pertussis* require droplet precautions. Unless we carefully identify which patients are co-infected with multiple agents, we may unknowingly use improper isolation or cohorting procedures. Because the frequency of virus co-infection is so high, we encourage testing for alternative respiratory pathogens even if a viral rapid diagnosis has already been established. Our results do not support routine testing or screening for *Bordetella* infection in young children hospitalized with febrile respiratory illness.

Acknowledgments

Funding

The authors disclosed receipt of the following financial support for the research and/or authorship of this article:

Funding of this project was provided by Central New York Children's Miracle Network (IRB#5553 to JBD) and NIAID Division of Intramural Research (Grant No. Z01-AI000943 to HFR).

References

1. Henrickson KJ. Advances in the laboratory diagnosis of viral respiratory disease. Pediatr Infect Dis J. 2004; 23(1 Suppl):S6–S10. [PubMed: 14730264]
2. Jennings LC, Anderson TP, Werno AM, Beynon KA, Murdoch DR. Viral etiology of acute respiratory tract infections in children presenting to the hospital. Pediatr Infect Dis J. 2004; 23:1003–1007. [PubMed: 15545854]
3. Semple MG, Cowell A, Dove W, et al. Dual infection of infants by human metapneumovirus and human respiratory syncytial virus is strongly associated with severe bronchiolitis. J Infect Dis. 2005; 191:382–386. [PubMed: 15633097]
4. Richard N, Komurian-Pradel F, Javouhey E, et al. The impact of dual viral infection in infants admitted to a pediatric intensive care unit associated with severe bronchiolitis. Pediatr Infect Dis J. 2008; 27:213–217. [PubMed: 18277932]
5. Greensill J, McNamara PS, Dove W, Flanagan B, Smyth RL, Hart CA. Human metapneumovirus in severe respiratory syncytial virus bronchiolitis. Emerg Infect Dis. 2003; 9:372–375. [PubMed: 12643835]
6. Kusel MMH, de Klerk NH, Holt PG, Kebadze T, Johnston SL, Sly PD. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life. Pediatr Infect Dis J. 2006; 25:680–686. [PubMed: 16874165]
7. Williams JV, Edwards KM, Weinberg GA, et al. Population-based incidence of human metapneumovirus infection among hospitalized children. J Infect Dis. 2010; 201:1890–1898. [PubMed: 20446850]
8. Stempel HE, Martin ET, Kuypers J, Englund JA, Zerr DM. Multiple viral respiratory pathogens in children with bronchiolitis. Acta Paediatr. 2009; 98:123–126. [PubMed: 18785966]
9. Peng D, Zhao D, Liu J, et al. Multipathogen infections in hospitalized children with acute respiratory infections. Virol J. 2009; 6:155–162. [PubMed: 19788746]
10. Aberle JH, Aberle SW, Pracher E, Hutter HP, Kundi M, Popow-Kraupp T. Single versus dual respiratory virus infections in hospitalized infants. Pediatr Infect Dis J. 2005; 24:605–610. [PubMed: 15999001]
11. Calvo C, Garcia-Garcia ML, Blanco C, et al. Multiple simultaneous viral infections in infants with acute respiratory tract infections in Spain. J Clin Virol. 2008; 42:268–272. [PubMed: 18455958]
12. Papadopoulos NG, Moustaki M, Tsolia M, et al. Association of rhinovirus infection with increased disease severity in acute bronchiolitis. Am J Respir Crit Care Med. 2002; 165:1285–1289. [PubMed: 11991880]

13. Allander T, Jartti T, Gupta S, et al. Human bocavirus and acute wheezing in children. Clin Infect Dis. 2007; 44:904–910. [PubMed: 17342639]
14. Nelson WL, Hopkins RS, Roe MH, Glode MP. Simultaneous infection with *Bordetella pertussis* and respiratory syncytial virus in hospitalized children. Pediatr Infect Dis J. 1986; 5:540–544.
15. Korppi M, Hiltunen J. Pertussis is common in nonvaccinated infants hospitalized for respiratory syncytial virus infection. Pediatr Infect Dis J. 2007; 26:3316–3318.
16. Marguet C, Lubrano M, Gueudin M, et al. In very young infants severity of acute bronchiolitis depends on carried viruses. PLoS ONE. 2009; 4(2):e4596. [PubMed: 19240806]
17. Reijans M, Dingemans G, Klaassen CH, et al. Respi-Finder: a new multiparameter test to differentially identify fifteen respiratory viruses. J Clin Microbiol. 2008; 46:1232–1240. [PubMed: 18256230]
18. Ginocchio CC. Detection of respiratory viruses using nonmolecular based methods. J Clin Virol. 2007; 40(Suppl 1):S11–S14. [PubMed: 18162248]
19. Schutzbank, TE.; McGuire, R.; Scholl, DR. Immunofluorescence. In: Spector, S.; Hodinka, RL.; Young, SA.; Wiedbrauk, DL., editors. Clinical Virology Manual. 4th ed.. Washington, DC: ASM Press; 2009. p. 77-88.
20. Fabbiani M, Terrosi C, Martorelli B, et al. Epidemiological and clinical study of viral respiratory tract infections in children from Italy. J Med Virol. 2009; 81:750–756. [PubMed: 19235872]
21. Canducci F, Debiaggi M, Sampaolo M, et al. Two-year prospective study of single infections and co-infections by respiratory syncytial virus and viruses identified recently in infants with acute respiratory disease. J Med Virol. 2008; 80:716–723. [PubMed: 18297694]
22. Esper F, Ou Z, Huang YT. Human coronaviruses are uncommon in patients with gastrointestinal illnesses. J Clin Virol. 2010; 48:131–133. [PubMed: 20362494]
23. Lai, MMC.; Holmes, KV. Coronaviridae: the viruses and their replication. In: Knipe, DM.; Howley, PM., editors. Field's Virology. 4th ed.. Philadelphia, PA: Lippincott Williams & Wilkins; 2001. p. 1163-1186.
24. Principi N, Bosis S, Esposito S. Effects of coronavirus infections in children. Emerg Infect Dis. 2010; 16:183–188. [PubMed: 20113545]
25. Bryant PA, Tebruegge M, Papadakis G, et al. Clinical and microbiological features associated with novel swineorigin influenza A pandemic 2009 (H1N1) virus in children. Pediatr Infect Dis J. 2010; 29:1–5. [PubMed: 19907356]
26. Bender JM, Ampofo K, Gesteland P, et al. Influenza virus infection in infants less than three months of age. Pediatr Infect Dis J. 2010; 29:6–91. [PubMed: 19915513]

Table 1

Viruses Identified as Single and Multiple Agent Infections

	n (%)
Patients tested	201 (100)
Positive viral tests	187 (93)
Patients with single viral agent	135 (67)
Patients with multiple viral agents	52 (26)
Single viral agents	135 (100)
Respiratory syncytial virus (RSV)	76 (56)
RSV A	38 (28)
RSV B	37 (27)
RSV not typed	1 (1)
Rhinovirus/enterovirus	23 (17)
Coronavirus	13 (10)
Coronavirus OC43	5 (4)
Coronavirus NL63	3 (2)
Coronavirus HKU1	3 (2)
Coronavirus 229E	2 (1)
Parainfluenza	12 (9)
Parainfluenza 1	4 (3)
Parainfluenza 2	0 (0)
Parainfluenza 3	1 (1)
Parainfluenza 4	7 (5)
Human metapneumovirus	9 (7)
Adenovirus	1 (1)
Influenza	1 (1)
Influenza A	1 (1)
Influenza B	0 (0)

Table 2

Dual Viral Infection Combinations Identified in 50 Patient Samples[a]

	Ad	Influenza	PIV	hMPV	CoV	R/E	RSV
RSV	2	0	1	1	4	20	1
R/E	4	0	5	3	4	NA	
CoV	0	1	0	2	0		
hMPV	0	1	1	NA			
PIV	0	0	0				
Influenza	0	0					
Ad	NA						

Abbreviations: Ad, adenoviruses; PIV, parainfluenza viruses 1, 2, 3, and 4; hMPV, human metapneumovirus; CoV, coronaviruses NL63, 229E, OC43, and HKU1; R/E, rhinoviruses/enteroviruses; RSV, respiratory syncytial viruses A and B; Influenza, influenza viruses A and B; NA, not applicable.

[a]The 2 patients who had 3 different viruses detected from their nasal samples are not included in this table.

Clin Pediatr (Phila). Author manuscript; available in PMC 2012 August 13.

Clin Pediatr (Phila). Author manuscript; available in PMC 2012 August 13.

Table 3

Virus Detected by Clinical Diagnosis[a]

Clinical Diagnosis	RSV	R/E	CoV	HMPV	PIV	Ad	Influenza
Bronchiolitis	101	46	11	15	11	5	1
Pneumonia	6	12	7	3	5	0	2
Croup	0	1	0	0	2	0	0
URI	2	2	1	1	1	0	0
Apnea	1	1	0	0	2	0	0
Gastroenteritis	0	1	4	0	0	1	0
Hepatitis	1	1	2	0	0	1	0
Meningitis	0	2	0	0	0	0	0
Viral syndrome	1	2	0	1	1	0	0

Abbreviations: Ad, adenoviruses; PIV, parainfluenza viruses 1, 2, 3, and 4; hMPV, human metapneumovirus; CoV, coronaviruses NL63, 229E, OC43, and HKU1; R/E, rhinoviruses/enteroviruses; RSV, respiratory syncytial viruses A and B; URI, upper respiratory tract infection.

[a]Some patients had multiple diagnoses.

Table 4

Demographic and Clinical Findings by Number of Viral Agents Detected

	Number of Viruses Detected; n (%)		
	1 (n = 135)	**>1 (n = 52)**	**0 (n = 14)**
Mean age (months)	4	3	2
Male	77 (57)	32 (62)	7 (50)
Underlying medical conditions[a]	26 (19)	9 (17)	1
Supplemental oxygen requirement	80 (59)	32 (62)	4
Intensive care unit stay	21 (16)	9 (17)	0
Mean hospital stay (days)	3.8	4.3	2.6
Gestational age			
Extreme prematurity (<28 wk)	3 (2%)	2 (4%)	0
Very premature (28–32 wk)	3 (2%)	1 (2%)	1
Prematurity (33–36 wk)	14 (10%)	16 (31%)	1

[a]Underlying conditions included prematurity, chronic lung disease, trisomy 21, and congenital heart disease.