THE EPIDEMIOLOGY OF RESPIRATORY VIRUSES IN HOSPITALIZED CHILDREN AND ADOLESCENTS DURING THE 2015-2016 RESPIRATORY SEASON

Leila Christina Sahni

The University of Texas School of Public Health, lcsahni@gmail.com

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by

LEILA CHRISTINA SAHNI, BS, MPH

APPROVED:

________________________________________
ERIC L BROWN, PHD

________________________________________
LU-YU HWANG, MD

________________________________________
DEJIAN LAI, PHD

________________________________________
PEDRO A PIEDRA, MD

________________________________________
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2018
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by

LEILA CHRISTINA SAHNI
MPH, The University of Texas School of Public Health, 2010
BS, The University of Houston, 2005

Presented to the Faculty of The University of Texas School of Public Health
in Partial Fulfillment
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for the Degree of

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THE UNIVERSITY OF TEXAS
SCHOOL OF PUBLIC HEALTH
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Respiratory diseases are an important contributor to global morbidity and mortality, causing more than 2.5 million deaths each year. These diseases are a leading cause of death in children less than 5 years of age and disproportionately affect children in low- and middle-income countries, where poverty, malnutrition, and lack of access to care result in increased risk of infection and death. Although the burden of disease is lower in the United States, respiratory disease, in particular acute respiratory illness (ARI) caused by viruses, remains a substantial cause of morbidity. The epidemiology of many of the commonly detected respiratory viruses (ex. respiratory syncytial virus, influenza virus, rhinovirus/enterovirus, human metapneumovirus, and parainfluenza virus) has been well-described in children <5 years of age where disease burden is highest, but less is known about the contribution of these viruses in children ≥5 years of age. Emerging respiratory viruses, for which little to no population immunity exists, have the potential to substantially impact children’s health globally. Identification and characterization of emerging respiratory viruses is a public health priority.
This dissertation analyzed data collected by the New Vaccine Surveillance Network (NVSN), a network of 7 pediatric hospitals established to conduct active, population-based surveillance for ARI and acute gastroenteritis. The first manuscript describes the epidemiology of well-recognized respiratory viruses in children 5-17 years of age hospitalized with ARI, with an emphasis on symptoms associated with infection treatment received during hospitalization, and clinical outcomes. Although these viruses have been well characterized in younger pediatric populations, data are lacking for older children. The second manuscript describes the detection of 2 novel respiratory viruses, enterovirus D68 and bocavirus, and compares the clinical course of infection with these viruses to established respiratory viruses.

These manuscripts add to the existing literature of pediatric respiratory viruses. Their findings fill the knowledge gap about spectrum of disease associated with well recognized respiratory viruses in older children, who have not traditionally been included in prior studies, and provide valuable information about two emerging respiratory pathogens. This research may identify priority target groups for prevention and treatment strategies and could inform the development of new vaccines and treatments.
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BACKGROUND

Acute respiratory illness (ARI)

Globally, respiratory diseases are a major course of morbidity and mortality in individuals of all ages annually, with an estimated 292 million episodes resulting in 2.74 million deaths in 2015.\(^1\)\(^2\) Although respiratory illnesses affect individuals of all ages, they represent the fifth-leading cause of death in children <5 years of age and disproportionately occur in low-income countries, where poverty, malnutrition, exposure to environmental pollutants, and lack of access to care place children at increased risk of infection and death.\(^3\)

In these countries, effective prevention and therapeutic interventions, such as the promotion of breastfeeding, adoption of *Haemophilus influenzae* type b and pneumococcal conjugate vaccines, and antibiotic therapy, have been identified, which, if implemented, would reduce morbidity and mortality associated with respiratory infections.\(^3\)-\(^6\)

Despite the reduced disease burden compared to many low- and middle-income countries, acute respiratory illnesses (ARI) remain a substantial cause of pediatric morbidity and mortality in the United States (U.S.).\(^1\)\(^7\) In temperate climates, many respiratory viruses cause epidemics that follow a temporal pattern, occurring primarily in the fall and winter months in the northern hemisphere in what is commonly referred to as the “respiratory season,” but some circulate endemically, causing illness year-round.\(^8\) Infections with respiratory viruses can be classified as either upper respiratory tract infections (URTI), which are characterized by nasal congestion, rhinorrhea, cough, and sore throat, or lower respiratory tract infections (LRTI), which include rapid breathing (tachypnea), increased work of
breathing characterized by chest wall retraction, rales and wheezing, hypoxia, cyanosis, and shortness of breath.9

**Respiratory syncytial virus (RSV)**

Respiratory syncytial virus (RSV) is a non-segmented, single stranded, negative-sense, enveloped RNA virus in the *Pneumoviridae* family. The virus genome contains 10 genes that encode 11 structural and non-structural proteins; of the 7 structural proteins, 3 are located in the viral envelope.10,11 The attachment (G) protein is involved in early attachment to the host cell, the fusion (F) protein mediates viral attachment to the host cell, fusion of cell membranes, and cell entry, and, while the role of the small hydrophobic (SH) protein is less well studied, it is thought to be involved in cell fusion and membrane permeability.10,12-14 Viruses are categorized into two subgroups (A and B) based on variability in the G-protein, with additional genotypes existing in each group.15

Globally, an estimated 33 million cases of RSV occur each year in children <5 years of age, resulting in approximately 3 million hospitalizations and more than 60,000 deaths.16 In the U.S., RSV is the primary cause of hospitalization among children <1 year of age, with rates highest among children <6 months.17 Rates of disease peak at 2-3 months of age, as maternal antibodies to RSV wane, and decrease with age.17,18 Universal infection occurs by 2 years of age and reinfection throughout childhood and among adults is common.17 The virus is the leading cause of bronchiolitis and pneumonia in young children, and severe infection with RSV during infancy has been linked to recurrent wheezing and asthma in later childhood.19,20 RSV bronchiolitis and pneumonia are characterized by wheezing and rales, chest wall retraction, fever, difficulty breathing and feeding, and hypoxia.
RSV follows a distinct seasonal pattern lasting 2-3 months each year; the season typically begins in October and ends in May with some regional differences. Treatment for RSV is limited to supportive care. A monoclonal antibody, palivizumab, is recommended for prophylactic administration for prevention of RSV to infants who are at particularly high-risk for severe disease; this includes infants born before 29 weeks gestation, infants born before 32 weeks gestation with chronic lung disease, and infants <12 months with specific, severe congenital heart disease. Vaccines against RSV are in development, but none are currently licensed. Because the highest burden of RSV disease occurs in infants <3 months of age, maternal immunization has emerged as a potential strategy to prevent disease in young infants, and a candidate vaccine targeting the F protein, which has been demonstrated to be immunogenic and safe, is being evaluated in an ongoing Phase III maternal vaccination trial to protect their young infants.

**Influenza virus**

Influenza occurs less frequently in children than RSV, but remains a substantial cause of ARI in children. Hospitalization rates due to influenza are estimated to be 0.9 per 1,000 in children <5 years and 4.5 per 1,000 in children <6 months. Annual outbreaks of influenza occur during winter months, beginning as early as October and typically peaking by February, and ending as late as May. Influenza viruses are classified by subtype (influenza A viruses: H1N1, H3N2, etc) or lineage (influenza B viruses: B-Victoria and B-Yamagata). In children the influenza viruses can be equally severe while in adults the severity of disease varies by infecting strain with H3N2 causing the highest mortality in older adults. A third type of influenza virus, influenza C, also causes ARI, but occurs less frequently.
human influenza A subtypes that circulate in the U.S. and globally are H3N2 and pandemic H1N1; the influenza B lineages (Yamagata and Victoria) that circulate in the U.S are also found globally. Individuals infected with influenza commonly experience fever, cough, rhinorrhea, and myalgias. Several antiviral drugs are available to treat individuals infected with influenza and also as prophylaxis for those who are exposed to an infected individual.29 Vaccines against influenza containing two influenza A strains and one or two influenza B strains are manufactured each year and recommended for all individuals >6 months of age in the U.S; the selection of strains for inclusion in the vaccine varies by the anticipated predominant circulating viruses each year. Although effectiveness varies and is dependent on how well the strains selected for inclusion match circulating viruses, vaccination remains the most effective prevention strategy.30

**Human metapneumovirus (HMPV)**

Human metapneumovirus (HMPV) was recently discovered in 2001 through a retrospective assessment of respiratory samples obtained from children <2 years of age in the Netherlands.31 Additional studies conducted globally suggest that HMPV has been circulating since the 1950s.32 HMPV disease follows a similar course to RSV; bronchiolitis, hypoxia, and fever are frequently reported, but HMPV typically occurs in older children and results in milder illness than RSV.33 HMPV infections occur most commonly in late winter and spring. Serologic studies suggest that near-universal infection occurs by 5 years of age; however, hospitalization rates for HMPV are substantially lower than for RSV. In the U.S., 1 per 1,000 in children <5 years and up to 4 per 1,000 in children <6 months of age are hospitalized due to HMPV.33,34 Treatment for HMPV is limited to supportive care, and
although preclinical trials of candidate vaccines have been conducted in animals, none are close to human trials or licensure.\textsuperscript{35}

\textbf{Parainfluenza virus (PIV)}

Parainfluenza viruses (PIV) are a common cause of lower respiratory tract infections, accounting for up to 17\% of hospitalizations in children <5 years of age. The burden of disease is highest among children <6 months of age: 4.6 children per 1,000 are hospitalized with parainfluenza, compared with 1.2 children per 1,000 <5 years of age.\textsuperscript{36} Four serotypes (PIV-1-4) cause disease in children and the viruses, particularly PIV-1, are the major cause of croup in young children.\textsuperscript{37} Parainfluenza viruses follow distinct seasonal patterns; PIV-3 accounts for >50\% of PIV disease and is associated with annual outbreaks of bronchiolitis and pneumonia occurring during spring and summer months, while PIV-1 and, to a lesser extent, PIV-2 are associated with disease occurring in the fall.\textsuperscript{38,39} Parainfluenza virus 4 occurs less frequently and has not been well-characterized.\textsuperscript{36} An experimental antiviral for the treatment of PIV exists and has demonstrated effectiveness in immunosuppressed individuals, but is not available for routine clinical use.\textsuperscript{40,41} There is currently no licensed vaccine against PIVs, but development primarily targeting PIV-3 began in the 1960s. Several candidate vaccines have completed preclinical and animal studies and two have completed early phase trials in humans.\textsuperscript{42}

\textbf{Adenovirus}

Adenoviruses are an important contributor to febrile and respiratory illnesses in children, and are estimated to be responsible for up to 10\% of febrile illness in children <5 years of age.\textsuperscript{43} Adenoviruses most commonly cause respiratory illness, with symptoms
ranging from mild URTI to bronchiolitis and pneumonia, but may also result in systemic, disseminated disease occurring with bacterial superinfection. More than 60 serotypes have been identified; types 1-5, 7, 14, and 21 are associated with respiratory symptoms, while types 3 and 7 are associated with severe LRTI and disseminated disease. Additional symptoms include gastroenteritis, conjunctivitis, nephritis, and hepatitis. In children the most common adenovirus serotypes causing respiratory infections are serotypes 1, 2, 3, 5, 6 and 7. Outbreaks of acute respiratory illness due to serotypes 3, 4, 7, 21, and 14 have been reported among military recruits due to close quarters and crowding; vaccination of military personnel with a live, oral vaccine is now routine practice and has resulted in substantial disease reduction in this population. Antivirals have limited effectiveness against adenovirus and are restricted to use among immunosuppressed individuals.

**Rhinovirus**

Human rhinoviruses (HRV) were first discovered in the 1950s and are the pathogen most commonly associated with cold-like symptoms. HRV infections in children are common, and may be symptomatic or asymptomatic. Detection of HRV from asymptomatic individuals is more common in young children than adults, and is reported to range from 12-33% in healthy children <3 years and <5 years of age, respectively. Upper respiratory tract symptoms occur most frequently with HRV infection, although lower respiratory tract manifestations also occur, particularly among infants and patients with asthma. Disease occurs year-round, with peak incidence in the fall. Rhinoviruses are categorized in three groups: A, B, and C; HRV-C and HRV-A have been linked to more severe lower respiratory tract symptoms, including bronchiolitis and pneumonia, in children than HRV-B. As with
RSV, rhinovirus infections have been associated with recurrent wheezing and development of asthma later in childhood. No rhinovirus-specific treatment or prevention exists.

**Coronavirus**

Six coronaviruses (CoV) have been associated with disease in humans; 4 human coronaviruses (229E, NL63, OC43, and HKU1) and two zoonotic coronavirus that causes severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). Currently, there is no known SARS transmission anywhere in the world. The most recent human cases were reported from China in April 2004. MERS was initially detected in 2012 with the majority of cases reported from Saudi Arabia. The case-fatality proportion is approximately ~36%. Only two imported cases of MERS have been reported in the U.S. Similarly to rhinoviruses, the human coronaviruses (229E, NL63, OC43, and HKU1) are most commonly associated with upper respiratory symptoms, but the full spectrum of disease ranges from asymptomatic infection to severe lower respiratory tract symptoms. Coronaviruses follow a seasonal pattern, peaking during winter months in the northern hemisphere, with virus detection occurring year-round. The contribution of coronaviruses to pediatric ARI is unclear; several studies conducted in a variety of settings have found no difference in the detection rates of CoVs among hospitalized children and asymptomatic, healthy controls. There are no prevention strategies or intervention designed specifically to target human coronaviruses; treatment is limited to supportive care.

**Enterovirus**

More than 70 serotypes of non-polio enteroviruses have been identified globally, with >10 million infections estimated to occur in the U.S. alone. Enteroviruses occur most
commonly during summer and fall months, and most manifest as a mild, non-specific febrile illness.\textsuperscript{59,60} Enteroviruses are the cause of several syndromes, including herpangina and hand, foot, and mouth disease.\textsuperscript{61} Severe enterovirus infection disproportionally impacts infants and young children; enteroviruses are the leading cause of aseptic meningitis in children <5 years of age.\textsuperscript{62} Enteroviral infection in neonates can be particularly severe and is characterized by systemic infection resembling sepsis with multi-organ involvement.\textsuperscript{63} Most cases of enterovirus meningitis resolve without complications. In Asia, however, major outbreaks of enterovirus type 71 is associated with significant mortality in children and thus that survive frequently have residual neurologic deficits. In the U.S. regional and temporal outbreaks of enteroviruses occurs, including an outbreak of severe respiratory illness in children in 2014 associated with enterovirus D68 (EV-D68).\textsuperscript{64}

**Emerging respiratory pathogens**

Surveillance for emerging respiratory pathogens that have the potential to cause global epidemics is a public health priority.\textsuperscript{65} Increasing knowledge of recently detected, but poorly-characterized viruses will facilitate the rapid and timely identification of outbreaks of disease, and will also allow for increased understanding about the contribution of these viruses to respiratory illness. Two such respiratory viruses are enterovirus D68 and bocavirus.

**Enterovirus D68 (EV-D68)**

Enterovirus D68 was first identified in 1962 in children with severe respiratory tract infection. Individual cases and small, local outbreaks have since occurred, but these have been geographically limited.\textsuperscript{66} During August 2014 through January 2015, a large,
nationwide outbreak of EV-D68 associated with severe respiratory illness occurred in children, many of whom had a history of wheezing or asthma. Many children hospitalized with EV-D68 during this outbreak experienced difficulty breathing, hypoxia, and wheezing and required mechanical ventilation and/or admission to an intensive care unit (ICU). EV-D68 has been associated with neurological symptoms in a small number of individuals, including acute flaccid myelitis, and has been detected in cerebrospinal fluid and brain tissue samples obtained at autopsy from one child and in the blood of another child with acute flaccid paralysis. Although these suggest that neurological involvement with EV-D68 infection is possible, additional data are needed.

**Bocavirus**

Human bocavirus was first detected in Sweden in 2005 and has since been associated with a variety of respiratory symptoms in children, ranging from mild upper respiratory tract symptoms to severe lower respiratory tract manifestations. However, bocavirus is frequently detected in the presence of other respiratory viruses, estimates of coinfection range from 33-67%, and has been detected in up to 44% of asymptomatic children. Although bocavirus has been identified in up to 20% of children <2 years of age with ARI, its role in symptomatic disease is unclear and additional investigation is warranted.

**The New Vaccine Surveillance Network (NVSN)**

The New Vaccine Surveillance Network (NVSN) was established in 1999 to assess the impact of vaccines and vaccine policies. The network conducts population-based, active surveillance for acute gastroenteritis (AGE) and ARI at seven surveillance sites across the U.S. For the period covered in this dissertation, the seven locations were: Oakland,
California, Seattle, Washington, Houston, Texas, Kansas City, Missouri, Nashville, Tennessee, Cincinnati, Ohio, and Rochester, New York. In 2016, the Oakland site was replaced by Pittsburgh, Pennsylvania. The network conducted surveillance for ARI during 2000-2009, and then again from 2015 to the present, and has conducted continuous surveillance for AGE since 2006.

For the period included in this dissertation, children 0-17 years of age who were hospitalized at one of eight pediatric hospitals (UCSF Benioff Children’s Hospital Oakland in Oakland, California, Seattle Children’s Hospital in Seattle, Washington, Texas Children’s Hospital in Houston, Texas, Children’s Mercy Hospitals and Clinics in Kansas City, Missouri, Monroe Carell Jr. Children’s Hospital at Vanderbilt in Nashville, Tennessee, Cincinnati Children’s Hospital Medical Center in Cincinnati, Ohio, and Golisano Children’s Hospital and Rochester General Hospital in Rochester, New York) during November 1, 2015 through June 30th, 2016, with fever and/or respiratory diagnoses were offered participation. Upon obtaining informed consent, a 25-item questionnaire was administered to the participant’s parent/guardian, capturing information about participant demographic and sociodemographic factors, risk factors for respiratory illness, and symptoms and treatment received prior to hospitalization. After enrollment, a 23-item medical abstraction tool was completed for all participants. This captured information about the participant’s past medical history, clinical course (key physical examination findings, duration of stay, acuity of care, interventions received) during hospitalization, admission and discharge diagnoses, and results of relevant laboratory testing. Combined mid-turbinate nasal and throat swabs were obtained and were tested locally using molecular assays for a core set of respiratory viruses
(influenza, RSV, parainfluenza, human metapneumovirus, rhinovirus, enterovirus, enterovirus D68, and adenovirus); additional viral testing for bocavirus, coronavirus, and herpes simplex virus was conducted at some sites. Some sites also included molecular testing for selected bacteria (*Bordatella pertussis*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and *Legionella pneumophila*), although these results are not included in this dissertation.

While the primary objectives of the NVSN ARI component are to evaluate the impact of vaccines and vaccine policies, the network conducts surveillance for a wide range of respiratory pathogens that are not yet vaccine-preventable with the intent of generating robust, baseline estimates of disease burden that are population-based in anticipation of new vaccine development. Many of the estimates of disease burden in children <5 years of age presented earlier were generated from NVSN surveillance for respiratory pathogens that was conducted during 2000-2009. These data will permit post-introduction assessments of impact and effectiveness. Beyond determining incidence rates of respiratory pathogens, the comprehensive data available about risk factors for disease, past medical history, and clinical course allow for the characterization of pathogen-specific epidemiology occurring across the U.S., particularly among children >5 years of age who have not typically been included in assessments of respiratory illness.

**Public Health Significance**

Acute respiratory illnesses caused by viruses have been widely described in children <5 years of age, but data are lacking among children ages 5-17 years. This dissertation aims
to broaden the knowledge of the role of respiratory viruses in ARI in hospitalized children 5-17 years of age. Specifically, it will describe the clinical course of hospitalization among children with respiratory viruses, examine factors associated with viral infection, and assess the impact of clinical testing and provider knowledge of the etiology of disease on treatment and clinical outcome. These data will fill a much-needed knowledge gap about the full range of disease in children and adolescents of all ages, and particularly in older children who have not been well described. There currently exists insufficient information about the burden of disease in this population, the spectrum of illness severity and outcomes, and the contribution of comorbidities to an individual’s risk of disease and clinical course. Finally, this dissertation will characterize two emerging respiratory pathogens, bocavirus and enterovirus D68, in children of all ages and compare the clinical course of hospitalization with well-recognized respiratory viruses, such as influenza, RSV, rhinovirus, HMPV, and PIV. The full epidemiology of bocavirus and enterovirus D68 has not been described, and their role in respiratory illness and local outbreaks is unclear. Knowledge gained from this dissertation will aid in the development of treatment and prevention strategies, including vaccine development, and identification of priority groups for intervention.

Specific Aims

This dissertation proposes two aims to describe the epidemiology of respiratory viruses in hospitalized children and adolescents younger than 18 years of age at seven pediatric hospitals in the United States. Specifically, it will examine viruses associated with hospitalization in older pediatric patients ages 5-17 years of age, which have not been widely
characterized, as well as the emerging respiratory viruses bocavirus and enterovirus D68 in
children of all ages. The specific aims are:

1. To describe the clinical course of children ages 5-17 years who are hospitalized at 7
   New Vaccine Surveillance Network sites with acute respiratory illnesses and examine
   variations in diagnostic approach, treatment, and outcomes in children with and
   without a respiratory virus.

2. To characterize emerging respiratory pathogens, with a particular focus on
   enterovirus D68 and bocavirus, identified in children 0-17 years of age at New
   Vaccine Surveillance Network sites and compare clinical presentation, hospitalization
   course, and outcomes of enterovirus D68 and bocavirus single and co-infections with
   well-recognized respiratory viruses, such as influenza, RSV, rhinovirus, HMPV, and
   PIV.

METHODS

This dissertation is a secondary analysis of data obtained from patients hospitalized
with ARI during November 1, 2015 through June 30, 2016 at seven New Vaccine
Surveillance Network sites.

Study Setting

Participants were recruited from eight hospitals associated with the seven New
Vaccine Surveillance Network sites: UCSF Benioff Children’s Hospital Oakland in Oakland,
California, Seattle Children’s Hospital in Seattle, Washington, Texas Children’s Hospital in
Houston, Texas, Children’s Mercy Hospitals and Clinics in Kansas City, Missouri, Monroe
Carell Jr. Children’s Hospital at Vanderbilt in Nashville, Tennessee, Cincinnati Children’s Hospital Medical Center in Cincinnati, Ohio, and Golisano Children’s Hospital and Rochester General Hospital in Rochester, New York.

**Study Subjects**

Children were eligible to participate if they were hospitalized at one of the eight locations and met the following inclusion criteria: 1) patient age <18 years; 2) residence within defined catchment areas at each NVSN site; 3) hospitalization for fever and/or respiratory illness; 4) illness duration <14 days; and 5) consent for participation obtained from parent/guardian within 48 hours of hospital admission. Children not meeting these inclusion criteria were ineligible for participation. Recruitment was conducted at each site a minimum of 5 days each week. Hospital censuses were reviewed for children meeting eligibility criteria; trained personnel at each site approached the parent/guardian of each potentially eligible child, eligibility criteria were reviewed, and eligible children were offered participation. Consent procedures and enrollment were completed in English and Spanish; eligible children of a parent/guardian who did not speak English or Spanish were not enrolled. Consent was obtained from the parent/guardian of all participants; child assent was also obtained for participants ≥7 years.

**Data Collection**

After obtaining consent for study participation, a paper-based 25-question questionnaire was verbally administered to the participant’s parent/guardian. This questionnaire was administered in either English or Spanish, depending on the
parent/guardian’s preference, and captured information about participant demographic and sociodemographic factors, risk factors for respiratory illness, and symptoms and treatment received prior to hospitalization. If the participant was enrolled during influenza season, information was obtained about vaccine receipt and a release of medical information was completed to permit contacting participants’ vaccine providers to verify vaccine receipt. After enrollment, a 23-item medical abstraction tool was completed for all participants. This captured information about the participant’s past medical history, clinical course (key physical examination findings, duration of stay, acuity of care, interventions received) during hospitalization, admission and discharge diagnoses, and results of relevant laboratory testing.

Mid-turbinate nasal and throat swabs were obtained from each participant within 72 hours of hospital admission (within 24 hours of enrollment). Tracheal aspirates were obtained in lieu of nasal and throat swabs from intubated patients. Respiratory samples were collected using flocked nylon swabs, placed into universal transport media, and transported to laboratories at each site, where they were stored at 2-8°C for up to 72 hours before processing. Respiratory samples were tested at each of the sites using molecular assays for a core set of respiratory viruses (influenza, respiratory syncytial virus (RSV), parainfluenza, human metapneumovirus, rhinovirus, enterovirus, enterovirus D68, and adenovirus); additional testing for bocavirus, coronavirus, herpes simplex virus, Bordatella pertussis, Mycoplasma pneumoniae, Chlamydophila pneumoniae, and Legionella pneumophila was conducted at some sites.
Data Analysis

Specific Aim 1

Pearson’s $\chi^2$, Fisher’s exact, and Kruskal-Wallis tests (categorical variables) and Wilcoxon rank sum test (continuous variables) were used to examine differences in sociodemographic factors, presenting symptoms, and treatment and clinical course in children with influenza, RSV, rhinovirus/enterovirus, HMPV, and PIV types 1-3 (henceforth referred to as “core respiratory viruses”) and also in children with no virus identified. Multivariable logistic regression was used to estimate the odds of virus detection after controlling for variables with clinical relevance and those significant at $p \leq 0.01$ in univariable analysis. When single infections only were analyzed; participants from whom multiple viruses were detected were excluded from analyses.

Specific Aim 2

Pearson’s $\chi^2$, Fisher’s exact, and Kruskal-Wallis tests (categorical variables) and Wilcoxon rank sum test (continuous variables) were used to examine differences in sociodemographic factors, presenting symptoms, and treatment and clinical course in children with EV-D68 and bocavirus infections compared with children infected with influenza, RSV, rhinovirus/enterovirus (excluding EV-D68), HMPV, or PIV types 1-3 (referred to as “core respiratory viruses”) infections or no virus detected. With the exception of bocavirus, only single infections were included; participants from whom multiple viruses were detected were excluded from analyses.
**Human Subjects Considerations**

Data analyzed include information from the questionnaire administered to each participant’s parent/guardian and data obtained through medical record abstraction, which captures information about participant demographic and sociodemographic factors, risk factors for respiratory illness, and clinical course of hospitalization. The results of respiratory viruses testing on mid-turbinate, throat, and tracheal aspirates were also analyzed.

Participants were assigned a subject identification number at enrollment and all study data were entered into a secure, encrypted online database managed by the Centers for Disease Control and Prevention. Patient identifiers are maintained at each study site and are not shared with non-local investigators.

The data analyzed to complete this dissertation were collected through the New Vaccine Surveillance Network. Institutional review board (IRB) approval was obtained from the Centers for Disease Control and Prevention, Baylor College of Medicine (representing the Texas NVSN site; protocol H-37691), and the remaining six study sites. In addition, this protocol was reviewed by the UTHealth IRB (protocol HSC-SPH-18-0686) and approved via reciprocity agreement with Baylor College of Medicine (Appendix A).
Abstract

Background: Moderate to severe acute respiratory illnesses (MSARI) occur less frequently in children ≥5 years than in children <5 years of age, but remain an important cause of morbidity. Knowledge of the epidemiology of respiratory viruses in MSARI has implications for treatment and prevention strategies.

Methods: We conducted prospective, active surveillance in children 5-17 years of age who were hospitalized at 7 New Vaccine Surveillance Network locations between November 1, 2015 and June 30, 2016 with MSARI. Sociodemographic information, respiratory illness risk factors, and symptoms were obtained from the parent/guardian at enrollment and medical record abstraction was performed. Respiratory samples were collected and tested using molecular assays for respiratory viruses, including influenza A and B, respiratory syncytial virus (RSV), rhinovirus/enterovirus, human metapneumovirus (HMPV), and parainfluenza viruses (PIV).

Results: Respiratory samples were tested from 880 participants; 382 (43.41%) were negative for all viruses while 498 (56.59%) were positive for any virus. Rhinovirus/enterovirus was most commonly detected in 36.94% (n=324) of participants, followed by influenza (6.04%, n=53), RSV (5.82%, n=51), HMPV (4.80%, n=42), and PIV (1.60%, n=14). Children with virus-positive MSARI more commonly presented with cough,
dyspnea, wheezing, fatigue, and nasal congestion than virus-negative children, and more frequently required supplemental oxygen during hospitalization. Differences were observed in presenting symptoms and clinical course by the virus identified. Most virus-positive children had a chronic lung or developmental comorbidity.

**Conclusions:** Children 5 years of age and older with lung and developmental health conditions are at increased risk for virus-positive MSARI in the hospital setting. Influenza vaccine for the prevention of virus-related MSARI is the only strategy we currently have for this age group. Clinical course and outcomes in children ≥5 years by viral etiology were similar to children <5 years of age.

**Background**

In the US, diseases of the respiratory systems, including asthma and pneumonia, account for 40% of hospital discharge diagnoses among children ages <5 years of age. The burden of acute respiratory illness (ARI) in the hospital (moderate to severe ARI) and outpatient setting and viral etiology of respiratory disease for children <5 years has been described by the New Vaccine Surveillance Network (NVSN). The NVSN was established by the Centers for Disease Control and Prevention (CDC) in 1999 to conduct prospective, population-based, active surveillance for ARI at children’s hospitals across the U.S. Admission rates for MSARI and/or fever were calculated to be 180 per 100,000 using NVSN data. A specific respiratory virus was identified in 61% of hospitalized children; most commonly respiratory syncytial virus (RSV) (20% of patients), parainfluenza viruses (7% of patients), and influenza (3% of patients). Other viruses identified included adenovirus,
human metapneumovirus (HMPV), and picornaviruses (primarily rhinoviruses). Also using NVSN data, hospitalization rates for children <5 years of age have been estimated at 4.8 per 1,000 for rhinoviruses, 3 per 1,000 for RSV, 1.02 per 1,000 for parainfluenza viruses (PIVs), 1 per 1,000 for HMPV, and 0.9 per 1,000 for influenza. When comparing symptoms and treatment by virus, children with influenza were more likely to present with fever, rule-out sepsis or febrile seizure, while children with RSV were more likely to require supplemental oxygen. Children with rhinovirus were more likely to present with wheezing and/or asthma exacerbation, while children with HMPV more typically presented with pneumonia and bronchiolitis. More recently, analysis of NVSN surveillance data from November 1—June 30, 2016 for children <5 years of age revealed that RSV was the most prominent virus detected in this age group between November and March; during the remaining portions of the year, rhinovirus predominated.

Similar to children <5 years, respiratory diseases account for 25% of discharge diagnoses in children ≥5 years. Although this accounts for a smaller proportion of hospitalizations in this age group, respiratory illness remains a significant contributor to overall disease. To date, the overall viral etiologies of MSARI in this age group have not been well described and most analyses have focused on the etiology of hospitalized children with community-acquired pneumonia. In a 3-year, prospective study in Finland of hospitalized children with community-acquired pneumonia, a viral etiology was identified in 37% of children, with RSV detected in 11% of children >5 years of age. In a prospective analysis of hospitalized children with radiographically-confirmed lower respiratory tract infections in the U.S., 75% of children >5 years had a detectable pathogen, of which
approximately 15% were single viral infections and 20% were mixed viral and bacterial infection. In a similar prospective study in Greece among children 5-14 years of age who were hospitalized with community-acquired pneumonia, one or more viruses were detected in 65% of patients. Among the viral pathogens detected in patients in this cohort, rhinovirus was found in 45% of cases, while adenovirus, PIV, influenza, RSV and HMPV were detected less commonly. Similarly, a study of community-acquired pneumonia in children <18 years of age conducted at 3 children’s hospitals in the U.S. identified a viral cause of illness in 30-40% of children ≥5 years of age. Rhinoviruses predominated in these children, accounting for approximately 25%-40% of viral infections, and occurred throughout the study period.

As previously described, NVSN conducts surveillance for a wide range of respiratory pathogens in children hospitalized with MSARI at seven sites across the US. Although this network has provided much of the available data for children <5 years of age, children ≥5 years have not typically been included in assessments of respiratory illness. The purpose of this study is to describe the viral etiology and clinical course of hospitalized children ages 5-17 years with MSARI.

Methods

Patient Enrollment

Prospective surveillance for hospitalized children less than 18 years of age with MSARI was conducted at all 7 NVSN surveillance sites (Oakland, California, Seattle, Washington, Houston, Texas, Kansas City, Missouri, Nashville, Tennessee, Cincinnati, Ohio, and Rochester, New York) between November 1, 2015 and June 30, 2016 using standardized
protocols. Children were eligible to participate if they were hospitalized with an admission
diagnosis of fever and/or ARI and met the following inclusion criteria: 1) residence within
defined catchment areas at each NVSN site; 2) illness duration <14 days; and 3) consent for
participation obtained from the parent/guardian within 48 hours of hospital admission.
Children who did not meet all inclusion criteria, those with fever and neutropenia (ANC
<500/μL), newborns hospitalized since birth, patients transferred from another hospital after
an admission of >48 hours, or those admitted <5 days after a prior hospitalization were
ineligible to participate. This analysis is restricted to hospitalized children 5 years to less than
18 years of age.

Surveillance was conducted at each site a minimum of 5 days per week. Hospital
censuses were reviewed for hospitalized children meeting eligibility criteria; trained
personnel approached the parent/guardian of each potentially eligible child, eligibility criteria
were reviewed, and eligible children were offered participation. Written informed consent
was obtained from the parent/guardian in English and/or Spanish; child assent was obtained
from hospitalized children ≥7 years of age. Institutional review board approval was obtained
from the CDC and each of the 7 surveillance sites.

**Data and Specimen Collection**

A questionnaire was administered to the parent/guardian at the time of enrollment that
captured sociodemographic information, respiratory illness risk factors, past medical history,
and symptoms and treatment received prior to hospitalization. Medical record abstraction to
capture participants’ past medical history, clinical course, and results of relevant laboratory
testing was completed after enrollment for all participants. Past medical history captured
information about chronic medical conditions, such as lung disease (including asthma and bronchopulmonary dysplasia), heart disease (including congenital heart disease), kidney disease, genetic/metabolic conditions, neurologic/neuromuscular disease, sickle cell disease, and immunodeficiency.

Mid-turbinate nasal and throat swabs were obtained within 72 hours of hospital admission; tracheal aspirates were obtained in lieu of nasal and throat swabs from some intubated patients on mechanical ventilation. Respiratory samples were tested using molecular assays for influenza A & B, RSV, PIV 1-3, HMPV, rhinovirus/enterovirus, enterovirus D68, and adenovirus at each site; additional bacterial testing and viral testing for influenza C (n=2 of 7 sites), PIV type 4 (n=6 of 7 sites), human bocavirus type 1 (n=4 of 7 sites), and coronavirus (n=6 of 7 sites) occurred at some sites.

**Data Analysis**

Pearson’s $\chi^2$, Fisher’s exact, and Kruskal-Wallis tests (categorical variables) and Wilcoxon rank sum test (continuous variables) were used to examine differences in sociodemographic factors, presenting symptoms, and treatment and clinical course in children with influenza, RSV, rhinovirus/enterovirus, HMPV, and PIV types 1-3 (henceforth referred to as “core respiratory viruses”) and also in children with no virus identified. Multivariable logistic regression was used to estimate the odds of virus detection after controlling for variables with clinical relevance and those significant at $p \leq 0.01$ in univariable analysis. When single infections only were analyzed; participants from whom multiple viruses were detected were excluded from analyses. In addition, participants with positive blood or CSF cultures obtained as part of their clinical care or with positive bacterial
research testing were excluded. When participants were enrolled multiple times during the surveillance period, the first hospitalization was included in analyses and all others were excluded. All analyses were conducted using Stata 12 (StataCorp LP, College Station, TX, USA).

Results

A total of 1,616 eligible children 5-17 years of age were approached for enrollment at the 7 NVSN surveillance sites (Figure 1.1). Consent for participation was obtained for 925 (57.24%); the most common reasons for non-enrollment were declination by the parent/guardian (n=299; 43.27%), no parent/guardian available to provide consent (n=230; 33.29%), and hospitalized children discharge occurred before enrollment could be offered (n=79; 11.43%). After exclusions, a total of 880 participants were included in analyses; 382 (43.41%) were negative for all viruses while 498 (56.59%) were positive for any virus (Table 1.1). Rhinovirus/enterovirus was most commonly detected in 36.94% (n=324) of hospitalized children with MSARI, followed by influenza virus and RSV in 6.04% (n=53) and 5.82% (n=51) of enrolled children, respectively.

Hospitalized children from whom respiratory viruses were identified (“virus-positive”) were younger than hospitalized children from whom no virus was identified (“virus-negative”) (8.18 years vs. 8.83 years p=0.008) and differences were observed by location of enrollment. Virus-positive children were less likely to have received antibiotics prior to hospitalization and had a shorter duration of illness at the time of hospitalization (Table 1.2). These children were significantly more likely to present with cough, dyspnea,
wheezing, fatigue, and nasal congestion than virus-negative children. On physical examination, hospitalized children positive for a respiratory virus were significantly more likely to have chest wall retractions than virus-negative children and had higher maximum respiration rates with lower minimum oxygen saturation. Virus-positive children more frequently required supplemental oxygen during their hospitalization than virus-negative children (57.95% vs. 49.09% p=0.007); no other statistically significant differences were observed in treatment, length of stay, or the proportion requiring ICU care.

**Core viruses**

Single infections with core respiratory viruses occurred in 434 of 498 (87.15%) hospitalized children with MSARI. Rhinovirus/enterovirus occurred most frequently in 69.12% (n=300) of single infections, followed by influenza virus (n=48; 11.06%) and RSV (n=41; 9.45%) (Figure 1.2). Rhinovirus/enterovirus peaked in November and again in April through June; influenza virus primarily occurred in January through April, while RSV occurred in November through March. Similarities in symptoms and outcomes were observed across respiratory viruses. Specifically, the median duration of illness at the time of hospitalization was 3-4 days regardless of the virus detected, and the majority of hospitalized children presented with cough (≥95%), fatigue (≥70%), and anorexia (≥53%) (Table 1.3). Similarly, cyanosis was uncommon in this population, occurring in less than 5% of hospitalized children, and differences were not observed in length of stay by the type of virus detected.

Influenza virus infection occurred in 48 of 434 (11.06%) hospitalized children with single viral infections. Compared with other core viruses, children with influenza virus
infection more frequently presented with fever (97.92% vs. 49.09%, p<0.001), but were less likely to report dyspnea (68.75% vs. 93.47%, p<0.001) or wheezing (51.06% vs. 83.25%, p<0.001). These children less commonly had chest wall retractions noted on physical examination (17.39% vs. 51.96%, p<0.001) but more frequently had altered mental status or confusion (6.25% vs. 1.31%, p=0.049). Children with influenza virus infection required supplemental oxygen less often during hospitalization than children with non-influenza viral MSARI (33.33% vs. 62.08%, p<0.001) and were less likely to require ICU admission (4.17% vs. 18.65%, p=0.008). Notably, influenza virus-positive children received antivirals more often (37.5% vs. 4.40%, p<0.001) and reported chronic medical conditions less frequently than hospitalized children with other respiratory virus infections (68.75% vs. 96.11%, p<0.001).

RSV single infections comprised 9.45% of single core viral ARI. As with rhinovirus/enterovirus and HMPV, the majority of children with RSV presented with wheezing. Although no differences were observed in the proportion of children requiring admission to an ICU, hospitalized children with RSV were less frequently admitted to an observation unit than children with other viral MSARI (4.88% vs. 20.36%, p=0.012).

Hospitalized children with rhinovirus/enterovirus infection more frequently presented with wheezing (87.58% vs. 61.83%, p<0.001) and/or had wheezing noted on physical exam (72.24% vs. 28.64%, p<0.001), but were less likely to report fever during their illness (41.14% vs. 84.33%, p<0.001) than hospitalized children infected with other respiratory viruses. The majority of children with rhinovirus/enterovirus infection were hospitalized with a diagnosis of asthma (63.67% p<0.001). Compared with hospitalized children infected with
other core viruses, children with rhinovirus/enterovirus infection required less diagnostics: they were less likely to have blood cultures (15.33% vs. 39.55%, p<0.001), chest x-rays (43.33% vs. 67.16%, p<0.001), or respiratory virus testing (30.87% vs. 61.65%, p<0.001).

HMPV infections accounted for 7.83% of single viral MSARI. Compared with other respiratory viruses, children with HMPV more frequently received medical attention prior to hospitalization (67.65% vs. 44.86%, p=0.011) that resulted in antibiotic prescription (26.47% vs. 8.56%, p=0.001). These hospitalized children experienced seizures more frequently (14.71% vs. 4.56%, p=0.028) and more commonly reported neurologic or neurodevelopmental conditions such as hydrocephalus, hypotonia, and microcephaly, than children with other viral ARI (47.06% vs. 13.75%, p<0.001).

PIV types 1-3 were least commonly detected in our population. These hospitalized children were similar to children infected with other core respiratory viruses; no differences were observed in presenting symptoms, physical examination findings, or treatment.

**Risk factors for viral ARI**

Significant associations between virus-positive MSARI and documentation of ≥1 chronic medical condition, receipt of antibiotics prior to presentation, duration of illness, presenting symptoms of cough, dyspnea, wheezing, and nasal congestion, and retractions, wheezing, maximum respiration rate, and minimum oxygen saturation noted on physical exam were observed in univariable analysis.

After adjusting for location of enrollment, participant age, sex, race/ethnicity, and insurance status, significant associations were observed between virus positive MSARI and documentation of ≥1 chronic medical condition, duration of illness, and presenting symptoms.
of cough and nasal congestion. Children positive for a core virus had 2.01 (95% CI: 1.09, 3.72) greater odds of having ≥1 chronic medical conditions compared with children with virus-negative MSARI. Odds ratios for duration of illness, cough, and nasal congestion were 0.78 (95% CI: 0.72, 0.85), 5.30 (2.28, 12.30), and 2.04 (1.39, 2.99), respectively.

Discussion

To our knowledge, this is the first prospective, multi-center active surveillance platform to characterize the viral etiologies and clinical outcomes of respiratory illness in hospitalized children ages 5-17 years. Our work is unique in that it includes all causes of respiratory illness among hospitalized children; other studies of respiratory illness in children ≥5 years have been limited to community-acquired pneumonia or lower respiratory tract infections.11-14 A viral cause of ARI was identified in >50% of children hospitalized with MSARI, which is lower than children <5 years of age, but suggests that respiratory viruses remain a substantial contributor to respiratory illness in older children.3,4 Consistent with other studies in children ≥5 years, rhinovirus/enterovirus predominated in our hospitalized children, accounting for approximately 37% of virus positive MSARI.13,14

We observed notable differences in presentation and clinical course by virus identified. Rhinovirus/enterovirus accounted for a higher proportion of virus-positive MSARI in our population of children 5-17 years of age than in children <5 years (36% vs. 25%), but presenting symptoms were similar.5 Two distinct periods of increased rhinovirus/enterovirus activity were observed in November and again in April through June in our population, but rhinovirus/enterovirus detection was high, occurring in >20% of hospitalized children, even
in months of lower detection. Our finding of nearly year-round detection of rhinoviruses/enterovirus in older children is consistent with existing studies.\textsuperscript{14,15} Participants hospitalized with rhinovirus/enterovirus MSARI commonly experienced wheezing, both at the time of presentation and also during their hospitalization, and the majority were admitted with acute asthma exacerbation. The association between wheezing and asthma exacerbation with rhinovirus infection has been well documented, and infection has been linked to persistent wheezing among some patients.\textsuperscript{5,16}

Unlike children with rhinovirus/enterovirus infection, those infected with an influenza virus had a distinctly different presentation. Fever occurred almost universally in this group and children with influenza virus infection were more likely to have altered mental status and/or confusion, but less likely to have moderate to severe respiratory symptoms (wheezing, dyspnea, retractions) or require supplemental oxygen. Importantly, hospitalized children with an influenza virus infection were significantly less likely to have received an influenza virus vaccine compared to other virus-positive children hospitalized with MSARI. This is reassuring as it suggests that, in our population, influenza virus vaccine prevented influenza virus-related hospitalization. Moreover, unlike the other core respiratory viruses, a third of children hospitalized with influenza virus infection were otherwise healthy with no chronic medical conditions. This finding is consistent with nationwide surveillance that has observed high rates of hospitalization and mortality in children with no pre-existing high-risk conditions.\textsuperscript{17}

HMPV was detected in relatively few participants, however seizures during hospitalization occurred more frequently in children infected with HMPV than those with
other respiratory viruses or virus-negative MSARI. Additionally, neurologic or neuromuscular chronic medical conditions were common in this group, occurring in almost 50% of hospitalized children with an HMPV infection. HMPV has been associated with central nervous system disorders, including seizures and encephalitis, in a small number of pediatric patients.\textsuperscript{18-20} Our findings provide additional support for this association and, consistent with other studies, suggest that children with underlying neurologic/neuromuscular disease may be at increased risk for neurologic complications from HMPV disease.\textsuperscript{21}

Although not statistically significant, hospitalized children infected with RSV were younger than children with other respiratory viruses, and commonly presented with wheezing, fever, and cough. These findings are consistent with the epidemiology of RSV infection in children <5 years of age.\textsuperscript{6} No differences were observed in symptoms, treatment or outcome in children with PIVs, likely due to the small proportion of children in whom these viruses were detected.

In regression analyses, duration of illness, presentation with cough and nasal congestion, and \( \geq 1 \) chronic medical condition were risk factors for virus-positive MSARI. Chronic medical conditions were more frequently observed in our population than in children <5 years of age in NVSN (data not presented), and with the exception of children with influenza, >95% of our population with virus-positive MSARI had \( \geq 1 \) co-morbidity. Consistent with children <5 years of age, children 5-17 years with virus-positive MSARI were more likely to require supplemental oxygen than children with virus-negative MSARI.\textsuperscript{10} Of note, rhinovirus/enterovirus associated asthma exacerbation accounted for the majority of virus-positive MSARI in our population; developing preventive measures and treatments
specific to rhinovirus/enterovirus is warranted, as these are likely to have a large effect on healthcare utilization in children with asthma.

This study had several limitations. First, enrollment occurred during a single respiratory season, resulting in low detection of some respiratory viruses. This likely impacted our ability to detect differences in participant characteristics and symptoms by viral etiology. Furthermore, 2015-2016 was a relatively mild influenza season, and our findings may not be generalizeable to seasons with more severe disease. Second, because variation in testing methodology occurred across the NVSN sites and not all sites tested for coronavirus, bocavirus, influenza C, and parainfluenza virus type 4, it is possible that misclassification may have occurred. Some hospitalized children at these sites with single viral infections may have been coinfected or may have been virus-positive when thought to be virus-negative. However, any misclassification that may have occurred is unlikely to have substantively impacted our findings given the relatively low detection of these non-core viruses by the sites that tested for them (Table 1). Third, most of the NVSN sites did not distinguish infection caused by rhinovirus from enteroviruses. Thus, the clinical descript and outcome associated with rhinovirus/enterovirus infection represents a combination of both entities. Fourth, limited information about medication usage (e.g. bronchodilator, antibiotic, and steroid) during hospitalization was obtained, so it was not possible to examine differences in the use of these treatments by viral detection. Finally, because this study included hospitalized patients only, we were unable to examine differences in severity of disease by inpatient and outpatient location of care.
Conclusion

In conclusion, respiratory viruses are an important, and potentially underestimated, contributor to moderate to severe respiratory illness resulting in hospitalization in children ≥5 years of age. Our findings suggest that presentation and clinical course in hospitalized children 5-17 years of age is consistent with children <5 years of age. However, older hospitalized children frequently had a high-risk medical condition that placed them at risk for MSARI following respiratory viral infection. Additional studies evaluating the burden of viral ARI and rates of hospitalization in children ≥5 years are needed.
Figure 1.1. Flow diagram of participant inclusion

Eligible
n = 1,816

Not enrolled
n = 891
- Parent/guardian declined to participate (n = 299)
- No parent/guardian available to provide consent (n = 209)
- Patient discharged before enrollment offered (n = 79)
- Parent/guardian speaks language other than English/Spanish (n = 30)
- Physician refused (n = 36)
- Parent/guardian speaks Spanish, no translator available (n = 12)
- Missed (n = 7)

Enrolled
n = 925

Excluded from statistical analysis
n = 36
- Enrolled multiple times during study period (n = 2)
- Positive blood or CSF culture (n = 5)
- Research testing positive for baricitinib (n = 29)

Included in statistical analysis
n = 889

Sample not obtained
n = 7

Respiratory sample obtained & tested
n = 882

All research testing noninvasive
n = 2

Positive for any virus
n = 496

Excluded
n = 35
Patients with co-infections

Positive for any single virus
n = 463

Excluded
n = 29
Patients with non-core viruses
- Adenovirus (n = 8)
- Coronavirus (n = 14)
- Parainfluenza virus 4 (n = 5)
- Bocavirus (n = 2)

Negative for all viruses
n = 382

Positive for any single core virus
n = 454
Figure 1.2. Hospitalizations and core virus etiology

- Number of Hospitalizations
- Month of Admission
- Proportion Positive

- Any Positive (%)
- Rhinovirus/Enterovirus (%)
- Influenza (%)
- RSV (%)
- HMPV (%)
- Parainfluenza 1 - 3 (%)

Legend:
- Hospitalizations
- Any Positive (%)
- Rhinovirus/Enterovirus (%)
- Influenza (%)
- RSV (%)
- HMPV (%)
- Parainfluenza 1 - 3 (%)
Table 1.1. Virus testing and detection by NVSN location

<table>
<thead>
<tr>
<th>Virus Type</th>
<th>N sites testing</th>
<th>N samples tested</th>
<th>N Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A &amp; B*</td>
<td>7</td>
<td>878</td>
<td>53</td>
<td>6.04</td>
</tr>
<tr>
<td>Influenza C</td>
<td>2</td>
<td>168</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>RSV*</td>
<td>7</td>
<td>875</td>
<td>51</td>
<td>5.82</td>
</tr>
<tr>
<td>Parainfluenza 1-3*</td>
<td>7</td>
<td>875</td>
<td>14</td>
<td>1.60</td>
</tr>
<tr>
<td>Parainfluenza 4</td>
<td>6</td>
<td>675</td>
<td>7</td>
<td>1.04</td>
</tr>
<tr>
<td>Human metapneumovirus*</td>
<td>7</td>
<td>875</td>
<td>42</td>
<td>4.80</td>
</tr>
<tr>
<td>Rhinovirus/enterovirus*</td>
<td>7</td>
<td>877</td>
<td>324</td>
<td>36.94</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>7</td>
<td>875</td>
<td>20</td>
<td>2.29</td>
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<tr>
<td>Coronavirus</td>
<td>6</td>
<td>773</td>
<td>23</td>
<td>2.98</td>
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<tr>
<td>Bocavirus</td>
<td>4</td>
<td>634</td>
<td>3</td>
<td>0.47</td>
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</table>

*Core respiratory virus that was uniformly tested at all NVSN sites
Table 1.2. Comparison of clinical characteristics and treatment by respiratory virus detection

<table>
<thead>
<tr>
<th>Prior Treatment</th>
<th>Virus detected N=498 (56.59%)</th>
<th>No virus detected N=382 (43.41%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N or Median % or Range</td>
<td>N or Median % or Range</td>
<td></td>
</tr>
<tr>
<td>Medical visit for illness</td>
<td>238 47.89 173 45.41</td>
<td>0.465</td>
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</tr>
<tr>
<td>Receipt of antibiotics</td>
<td>50 10.16 60 15.75</td>
<td>0.014</td>
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</tr>
<tr>
<td>Receipt of antivirals</td>
<td>3 0.61 6 1.57</td>
<td>0.144</td>
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</tr>
<tr>
<td>Receipt of influenza vaccine</td>
<td>302 63.71 222 60.00</td>
<td>0.270</td>
<td></td>
</tr>
<tr>
<td>Receipt of palivizumab</td>
<td>7 1.51 3 0.87</td>
<td>0.319</td>
<td></td>
</tr>
<tr>
<td>Exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daycare/preschool/school attendance</td>
<td>451 90.93 348 91.34</td>
<td>0.832</td>
<td></td>
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<tr>
<td>Smoking within the home</td>
<td>37 7.58 26 6.86</td>
<td>0.685</td>
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</tr>
<tr>
<td>Presenting Symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of illness at presentation; median (days; range)</td>
<td>3 &lt;1-13 4 1-14</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>280 56.45 208 54.88</td>
<td>0.643</td>
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<tr>
<td>Cough</td>
<td>479 96.38 347 91.08</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Dyspnea</td>
<td>448 90.51 300 78.95</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Wheezing</td>
<td>391 79.31 250 65.79</td>
<td>&lt;0.001</td>
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<tr>
<td>Apnea</td>
<td>27 5.48 20 5.25</td>
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<tr>
<td>Fatigue</td>
<td>387 79.63 275 73.73</td>
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<tr>
<td>Myalgia</td>
<td>147 34.35 104 29.80</td>
<td>0.178</td>
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</tr>
<tr>
<td>Nasal congestion</td>
<td>391 78.99 254 67.20</td>
<td>&lt;0.001</td>
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<tr>
<td>Sore throat</td>
<td>236 52.21 159 44.92</td>
<td>0.051</td>
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<tr>
<td>Anorexia</td>
<td>269 57.73 194 53.15</td>
<td>0.188</td>
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<tr>
<td>Vomiting</td>
<td>112 22.54 69 18.21</td>
<td>0.117</td>
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<tr>
<td>Diarrhea</td>
<td>62 12.50 53 14.06</td>
<td>0.500</td>
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</tr>
<tr>
<td>Seizure</td>
<td>30 6.10 23 6.04</td>
<td>0.970</td>
<td></td>
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<tr>
<td>Physical Examination Findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest indrawing/retractions</td>
<td>228 46.25 148 39.15</td>
<td>0.036</td>
<td></td>
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<tr>
<td>Cyanosis</td>
<td>9 1.82 12 3.15</td>
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<td>Wheezing</td>
<td>295 59.60 203 53.14</td>
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<td>11 2.23 13 3.40</td>
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<td>Maximum respiration rate/min, median (range)</td>
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<td>Minimum O₂ saturation, median (%) (range)</td>
<td>93 63-100 95 59-100</td>
<td>&lt;0.001</td>
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<td>Diagnostic Approach</td>
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<td></td>
<td></td>
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<tr>
<td>Respiratory panel</td>
<td>195 39.31 114 29.84</td>
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<td>Blood culture*</td>
<td>122 24.50 110 28.80</td>
<td>0.152</td>
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<td>CSF studies*</td>
<td>4 0.80 5 1.31</td>
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<td>Chest x-ray</td>
<td>252 50.70 205 53.66</td>
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<td>Treatment</td>
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<td>37 7.43 19 4.97</td>
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<td>Supplemental oxygen</td>
<td>288 57.95 188 49.09</td>
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<td>-- -- 1 0.26</td>
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<td>Death</td>
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<td>Admission Diagnosis</td>
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<td>Asthma</td>
<td>254 51.00 157 41.10</td>
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<tr>
<td>Chronic Medical Condition</td>
<td>Virus detected N=498 (56.59%)</td>
<td>No virus detected N=382 (43.41%)</td>
<td>p value</td>
</tr>
<tr>
<td>----------------------------------</td>
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<td>----------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>N or Median</td>
<td>% or Range</td>
<td>N or Median</td>
</tr>
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<td>Chronic Medical Condition</td>
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<td>Lung disease</td>
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<td>Developmental disorder</td>
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<td>Neurologic/neuromuscular disorder</td>
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<td>Heart disease</td>
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*Excludes children with positive blood or CSF cultures
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<tr>
<th>Study</th>
<th>14.3. Clinical characteristics of children with core respiratory viruses</th>
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<tr>
<td><strong>Patient Characteristics</strong></td>
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<td><strong>Age, median (years; range)</strong></td>
<td>9.03</td>
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<td>Male</td>
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<td><strong>Prior Treatment</strong></td>
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<td>Medical visit for illness</td>
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<tr>
<td>Receipt of antibiotics</td>
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<td>Receipt of antivirals</td>
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<td>Receipt of influenza vaccine</td>
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<td>Receipt of palivizumab</td>
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<td><strong>Exposure</strong></td>
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<tr>
<td>Daycare/preschool/school attendance</td>
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<td><strong>Presenting Symptoms</strong></td>
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<td>Duration of illness at presentation</td>
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<td>Fever</td>
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<td>Myalgia</td>
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<td><strong>Physical Examination Findings</strong></td>
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<td>Chest indrawing/retractions</td>
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<td>Cyanosis</td>
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<td>Maximum respiration rate/min, median (range)</td>
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<td>Minimum O₂ saturation (%)</td>
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<td><strong>Diagnostic Approach</strong></td>
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<td>Blood culture*</td>
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<td><strong>Treatment</strong></td>
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<td>Outcome</td>
<td>N or Median</td>
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<tr>
<td><strong>Outcome</strong></td>
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<td>Length of stay (days)</td>
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<td>Observation admission</td>
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<td>ICU admission</td>
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<td>Length of ICU stay (days)</td>
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<td><strong>Admission Diagnosis</strong></td>
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<td>26</td>
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<td>6</td>
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<td>8</td>
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<tr>
<td>disorder</td>
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*Excludes children with positive blood or CSF cultures*
References


Abstract

**Background:** Enterovirus D68 (EV-D68) has been sporadically detected in the United States since 2014 and has been associated with upper and lower respiratory tract illness of varying severity. Human bocavirus type 1 is a newly-discovered respiratory virus that has been detected in symptomatic and asymptomatic children. The spectrum of clinical disease and the impact of these 2 viruses in children have not been fully defined.

**Methods:** We conducted active, population-based surveillance for acute respiratory illness (ARI) in children hospitalized at 7 New Vaccine Surveillance Network (NVSN) locations during November 1, 2015 through June 30, 2016. Sociodemographic information, respiratory illness risk factors, and symptoms were obtained from the parent/guardian at enrollment and medical record abstraction was performed. Respiratory samples were obtained and tested using molecular assays for various respiratory viruses, including EV-D68 and bocavirus.

**Results:** EV-D68 and bocavirus were detected in 39 of 3,786 (1.03%) and 105 of 2,548 (4.12%) participants, respectively. EV-D68 was detected at 2 NVSN sites and occurred during spring and summer months only, suggestive of regional outbreaks. Children with EV-D68 were older (3.24 years vs. , had a shorter duration of illness at the time of hospitalization, and more frequently presented with wheezing and/or asthma exacerbation
than children with non-EV-D68 respiratory viruses. The majority (64%) of bocavirus infections occurred with other respiratory viruses. Children with bocavirus were younger than children with EV-D68, but were similar to children with non-bocavirus respiratory viruses.

**Conclusion:** EV-D68 and bocavirus are contributors to ARI in our population and were associated with both upper and lower respiratory tract disease of varied severity.

**Background**

Respiratory viruses, such as influenza and respiratory syncytial virus (RSV), are a leading cause of morbidity and mortality worldwide. Acute respiratory illnesses (ARI) remain a substantial contributor to pediatric morbidity and mortality attributable to infectious diseases in the United States (U.S.), and the burden of ARI disease is higher in low- and middle-income countries.\(^1\)\(^2\) Emerging respiratory pathogens for which little to no population immunity exists have the potential to substantially impact children’s health globally. For these reasons identification and characterization of emerging respiratory pathogens, such as enterovirus D68 and bocavirus, are a public health priority.\(^3\)

Enteroviruses (EV) belong to the Picornaviridae family, with four species (EV A-D) associated with disease in humans.\(^4\) More than 70 serotypes of non-polio enteroviruses have been identified globally, with >10 million infections estimated to occur in the U.S. alone.\(^5\) Enteroviruses circulate most commonly during summer and fall months, and cause a wide spectrum of illness.\(^4\)\(^6\)\(^7\) First identified in 1962, enterovirus D68 (EV-D68) has been associated with severe respiratory illness, including both upper and lower respiratory
infection and asthma exacerbation. A large, nationwide outbreak of EV-D68 occurred in 2014 among children, many of whom had a history of wheezing or asthma. Many children hospitalized with EV-D68 during this outbreak experienced difficulty breathing, hypoxia, and wheezing, and required mechanical ventilation and/or admission to an intensive care unit (ICU). Since 2014, EV-D68 has been detected sporadically in the U.S.; therefore, little is known about this emerging pathogen outside of the outbreak setting. Furthermore, although primarily a respiratory illness, EV-D68 has been detected in a small number of children who were diagnosed with acute flaccid myelitis. Given the spectrum of respiratory disease and potential association with acute flaccid myelitis, additional information about the clinical course and outcomes of EV-D68 infection in children is needed.

First detected in Sweden in 2005, human bocavirus type 1, which belongs to the Parvoviridae family, has been associated with a variety of respiratory symptoms in children, ranging from mild upper respiratory tract symptoms to severe lower respiratory tract manifestations. Bocavirus has been detected in 1%-11% of respiratory samples, and frequently occurs in the presence of other respiratory viruses. Estimates of coinfection range from 33-75%. Although bocavirus has been identified in up to 20% of children <2 years of age with ARI, its role in symptomatic disease is unclear and additional investigation is warranted to fully understand the spectrum of bocavirus disease in children.

The New Vaccine Surveillance Network (NVSN) was established by the Centers for Disease Control and Prevention (CDC) in 2000 to conduct population-based, active
surveillance for acute gastroenteritis and ARI at seven surveillance sites across the U.S. (Oakland, California, Seattle, Washington, Houston, Texas, Kansas City, Missouri, Nashville, Tennessee, Cincinnati, Ohio, and Rochester, New York). The purpose of this study is to describe EV-D68 and bocavirus infections in hospitalized children <18 years of age identified through active surveillance at NVSN sites and to compare the clinical course of hospitalization with well-recognized respiratory viruses, such as influenza, respiratory syncytial virus (RSV), rhinovirus, human metapneumovirus (HMPV), and parainfluenza virus (PIV) types 1-3.

Methods

Patient Enrollment

Prospective surveillance for patients <18 years of age hospitalized with acute respiratory illness was conducted at all NVSN surveillance sites during November 1, 2015 through June 30, 2016 using standardized protocols. Children were eligible to participate if they were hospitalized with an admission diagnosis of fever and/or acute respiratory illness and met the following inclusion criteria: 1) residence within defined catchment areas at each NVSN site; 2) illness duration <14 days; and 3) consent for participation obtained from the parent/guardian within 48 hours of hospital admission. Children who did not meet all inclusion criteria, those with fever and neutropenia (ANC <500/μL), newborns hospitalized since birth, patients transferred from another hospital after an admission of >48 hours, or those admitted <5 days after a prior hospitalization were ineligible to participate.
Surveillance was conducted at each NVSN site a minimum of 5 days per week. Hospital censuses were reviewed for children meeting eligibility criteria; trained personnel approached the parent/guardian of each potentially eligible child, eligibility criteria were reviewed, and eligible children were offered participation. Written informed consent was obtained from the parent/guardian in English and/or Spanish; child assent was obtained from participants ≥7 years of age. Institutional review board approval was obtained from the CDC and each of the seven surveillance sites.

*Data and Specimen Collection*

A questionnaire was administered to the parent/guardian at the time of enrollment that captured sociodemographic information, respiratory illness risk factors, past medical history, clinical symptoms, and treatment received prior to hospitalization. Medical record abstraction to capture participants’ past medical history, clinical course, and results of relevant laboratory testing was completed after enrollment for all participants.

Mid-turbinate nasal and throat swabs were obtained within 72 hours of hospital admission; tracheal aspirates were obtained in lieu of nasal and throat swabs from some intubated patients. Respiratory samples were tested using molecular assays for influenza, RSV, parainfluenza virus types 1-3, human metapneumovirus, rhinovirus/enterovirus, EV-D68, and adenovirus at each site; additional bacterial testing and viral testing for parainfluenza virus type 4, human bocavirus type 1, and coronavirus occurred at some sites. Testing methodology for EV-D68 varied by site; at 3 sites (California, Tennessee, New York) all samples were tested for EV-D68, while samples at the remaining 4 sites
(Washington, Missouri, Texas, Ohio) were tested using a two-step approach and only samples that were positive for rhinovirus and/or enterovirus were tested for EV-D68.

Data Analysis

Pearson’s $\chi^2$, Fisher’s exact, and Kruskal-Wallis tests (categorical variables) and Wilcoxon rank sum test (continuous variables) were used to examine differences in sociodemographic factors, presenting symptoms, and treatment and clinical course in children with EV-D68 and bocavirus infections compared with children infected with influenza, RSV, rhinovirus/enterovirus (excluding EV-D68), HMPV, or PIV types 1-3 (referred to as “core respiratory viruses” in this analysis) infections or no virus detected. With the exception of bocavirus, only single infections were included; participants from whom multiple viruses were detected were excluded from analyses. In addition, participants with positive blood or CSF cultures obtained as part of their clinical care or with positive bacterial research testing were excluded. When participants were enrolled multiple times during the surveillance period, the first hospitalization was included in analyses and all others were excluded. All analyses were conducted using Stata version 12 (StataCorp LP, College Station, TX, USA).

Results

A total of 6,342 eligible children ages 0-17 years of age were approached for enrollment at the 7 NVSN sites (Figure 2.1). Consent for participation was obtained for 3,926 (61.90%); the most common reasons for non-enrollment were declination by the parent/guardian (n=1,165; 48.22%), no parent/guardian available to provide consent (n=567;
23.47%), and patient discharge occurring before enrollment could be offered (n=362; 14.98%). One hundred and eleven participants were subsequently excluded from analyses. Of the remaining 3,786, 984 (25.99%) were negative for all viruses, while 2,802 (74.01%) were positive for ≥1 respiratory virus. Enterovirus D68 was detected in 39 of 3,786 participants with ARI (1.03%) while human bocavirus type 1 was detected in 105 of 2,548 (4.12%) participants with ARI who were tested for bocavirus at the California, Texas, Missouri, and Ohio sites. One participant was positive for both enterovirus D68 and bocavirus and was excluded from subsequent analyses.

**Enterovirus D68**

Enterovirus D68 (n=38) was detected in children enrolled from the Tennessee (n=2) and Texas (n=36) surveillance sites. The median age of participants was 3.24 years (range 0.64-11.54 years) and the majority were male (n=22; 59.78%), white (n=27; 71.05%), non-Hispanic (n=21; 55.26%), and publicly insured (n=20; 55.26%) (Table 2.1). The first case of EV-D68 was detected in a patient hospitalized in Texas in April; cases occurred throughout the remainder of the surveillance period, with half (n=19) occurring in May (Figure 2.2). Both cases of EV-D68 identified in Tennessee occurred in June, toward the end of the surveillance period. Children with EV-D68 frequently presented with fever (63.16%), cough (97.37%), dyspnea (100%), wheezing (78.95%), fatigue (88.89%), nasal congestion (76.32%), and anorexia (82.35%). Comorbidities were common in this population; 68.42% had ≥1 medical condition, more than half of whom had lung disease, including asthma and BPD.
Bocavirus

Bocavirus was detected in 104 participants enrolled from the California, Texas, Missouri, and Ohio surveillance sites. Of these, 37 (35.58%) were single infections while 67 (64.42%) were detected in the presence of other respiratory viruses, most commonly rhinoviruses/enterovirus and RSV (Figure 2.3). The median age of bocavirus-positive participants was 1.33 years, 65.38% (n=68) were male, 51.92% (n=54) were white, 70.19% (n=73) were non-Hispanic, and 60.58% (n=54) were publicly insured. Participants with bocavirus single infections were more likely to be Hispanic than those with coinfections (43.24% vs. 22.39%, respectively, p=0.026); no other differences in demographic factors were observed. Bocavirus occurred throughout the surveillance period with detection peaking in February and March. Bocavirus single infections were characterized by fever (78.38%), cough (97.30%), dyspnea (91.89%), wheezing (83.78%), fatigue (67.65%), nasal congestion (86.49%), and anorexia (78.38%). As with EV-D68, comorbidities were common, with 64.86% reporting ≥1 medical condition.

Comparison of enterovirus D68 and bocavirus single infections

Children with EV-D68 infections were older than children with bocavirus single infections (3.24 years vs. 1.49 years, respectively, p<0.001). Other sociodemographic factors were similar between groups, with the exception of race (Table 2.1). Children with EV-D68 more frequently attended daycare or school and had a shorter duration of illness at the time of hospitalization than children with bocavirus. Children with EV-D68 had lower minimum oxygen saturation levels noted on physical exam compared with children with bocavirus, but less commonly had blood cultures or respiratory testing performed as part of their clinical evaluation.
course. No differences were observed in wheezing or history of lung disease, but children with EV-D68 were more frequently admitted with asthma than children with bocavirus (47.37% vs. 21.62%, p=0.019).

**Comparison of enterovirus D68 and bocavirus to core respiratory viruses**

Differences in presentation were observed between children with enterovirus EV-D68 and core viruses (Table 2.2). Children with EV-D68 were older (p<0.001) and more likely to report dyspnea (p=0.018) than children with a core respiratory virus. These children had a shorter duration of illness at the time of hospitalization (2 days vs. 4 days, p<0.001), were less likely to have respiratory testing (p=0.025) and blood cultures (p=0.001) ordered, and more commonly had retractions (p=0.010) and wheezing (p=0.001) noted on physical exam. Supplemental oxygen use was more frequent among children infected with enterovirus D68 than children with core respiratory viruses (p<0.001). History of lung disease and admission diagnoses for asthma were more common among participants with enterovirus D68 (p=0.018 and p<0.001, respectively). Children with enterovirus D68 had shorter hospitalizations (median length of stay 1 day vs. 2 days, p<0.001) and more frequently were admitted for observation (p=0.041) than children with core respiratory viruses.

In contrast, children with bocavirus were more similar to children with core respiratory viruses. No differences were observed in presenting symptoms, physical examination findings, or treatment between participants with bocavirus single or coinfections and those with core viruses. Children with bocavirus single infections were more frequently admitted with pneumonia than children with non-viral ARI or ARI due to core respiratory viruses. In this study, bocavirus was most similar to RSV; other than older age at
presentation (median age 1.49 years vs. 0.50 years, p<0.001), no differences were observed between children with bocavirus and RSV, respectively.

Discussion

To our knowledge, this study represents the first multi-site assessment of EV-D68 and bocavirus in children hospitalized with respiratory illnesses who were identified through prospective, active surveillance. In addition, we describe characteristics of children with these emerging respiratory viruses in comparison with children hospitalized during the same time period with core respiratory viruses. We identified a viral cause of illness in 74% (n=2,802 of 3,786) of patients, of these 1% (n=39 of 2,786) had EV-D68 and 4% (n=105 of 2,548) had bocavirus.

In this study, children with EV-D68 were hospitalized during the spring and early summer months, which is consistent with reports suggesting that enteroviruses, particularly EV-D68, exhibit distinct seasonality. However, our cases occurred earlier than the late-summer and fall months in which EV-D68 has typically been previously reported and appear to represent a regional outbreak as all but 2 cases occurred in children hospitalized in Houston, Texas. The 2 cases detected in Tennessee occurred in June, at the end of the surveillance period. It is unclear if these cases represent the beginning of geographically diverse, widespread disease, as it is possible that additional cases may have been identified in later summer and fall months if surveillance had been extended later into the year, or if they are isolated occurrences.
Children with EV-D68 experienced more rapid progression of symptoms resulting in hospitalization than children with other viral causes of ARI. They commonly presented with cough, dyspnea, wheezing, and retractions, which have previously identified as hallmark symptoms of EV-D68 infection.\textsuperscript{11,23-25} Consistent with findings from other studies, asthma exacerbation was a frequent cause of hospitalization among children with EV-D68 in our study, and these children more frequently required supplemental oxygen than children with non-viral ARI and ARI caused by core viruses.\textsuperscript{9,11,23,26,27}

Once hospitalized, children with EV-D68 recovered and were discharged more rapidly than children with other respiratory viruses, and, unlike findings from prior studies, were less likely to require admission to an ICU.\textsuperscript{23,28} However, our study was conducted during a period of low EV-D68 circulation and did not coincide with a nationwide outbreak, which may have impacted our findings. Interestingly, children with EV-D68 were more likely to attend daycare or school for \textgreater 4 hours per week than children with ARI caused by a core virus, suggesting potential differences in exposure and transmission for EV-D68 compared with other respiratory viruses.

We detected bocavirus in 4\% of patients hospitalized with respiratory illness, which is similar to the proportion of children identified with parainfluenza virus types 1-3. In contrast to EV-D68, most bocavirus infections (64\%) occurred in combination with other respiratory viruses, which is consistent with findings from other studies.\textsuperscript{17,18,21} In this study, children with bocavirus single infections were similar to children with core respiratory viruses; no differences were observed in presenting symptoms and physical examination findings, treatment, or outcome. However, as has been previously reported, children with
bocavirus were more frequently admitted with pneumonia than children with other viral causes of ARI. In this population, we observed lower respiratory tract symptoms, including wheezing, associated with bocavirus that were difficult to distinguish from RSV. This similarity has been noted in prior assessments of bocavirus infection; however, differences in the proportion of patients with fever, pneumonia diagnoses, and antibiotic use that have been reported were not observed in our assessment.

Wheezing and asthma exacerbation are established sequelae of viral respiratory illnesses. The occurrence of wheezing during and after infection has been well-described for rhinoviruses and RSV; both EV-D68 and rhinoviruses belong to the Picornaviridae family, so it is not surprising that wheezing has been associated with EV-D68 in this and other studies. A similar relationship between bocavirus and wheezing has been reported in a smaller number of studies, and our findings provide additional support for this association.

This study had several limitations. It was conducted during a single respiratory season and did not include late summer and fall months when EV-D68 is known to circulate. For this reason, the EV-D68 cases identified may underestimate the magnitude of cases that occurred in 2015-2016. EV-D68 has been suggested as a potential cause of acute flaccid myelitis in a limited number of studies. However, because we did not capture information about neurologic signs and symptoms, we were unable to examine any potential relationship between EV-D68 and acute flaccid myelitis. Respiratory samples obtained from participants at 3 of the 7 surveillance sites were not tested for bocavirus, so it is possible that misclassification may have occurred. However, this is unlikely to have substantively
impacted our results due to the relatively low proportion of samples that were positive for bocavirus. No information about bronchodilator, antibiotic, or steroid use during hospitalization was collected, so it was not possible to examine differences in the use of these treatments. Finally, because this study included hospitalized patients only, we were unable to describe the full spectrum of disease of these viruses that may have been observed in outpatient settings.

Conclusion

In conclusion, EV-D68 and bocavirus were important contributors to respiratory illness in hospitalized children in this multi-site study. Given the high proportion of patients with EV-D68 who presented with wheezing and asthma exacerbation, follow-up studies to evaluate a potential relationship between infection and persistent wheezing are warranted. Continued surveillance for EV-D68 and bocavirus encompassing multiple respiratory seasons and a variety of clinical settings is needed to better understand the burden and impact of EV-D68 and bocavirus infections in children.
Figure 2.1. Flow diagram of participant inclusion

Eligible

$\begin{align*}
n = 6,342
\end{align*}$

Not enrolled

$\begin{align*}
n = 2,436
\end{align*}$

- Parent/guardian declined to participate ($n = 1,165$)
- No parent/guardian available to provide consent ($n = 567$)
- Patient discharged before enrollment offered ($n = 902$)
- Parent/guardian speaks Spanish, no interpreter available ($n = 150$)
- Physician refused ($n = 72$)
- Parent/guardian speaks language other than English/ Spanish ($n = 50$)
- Missed ($n = 40$)

Enrolled

$\begin{align*}
n = 3,926
\end{align*}$

Sample not obtained

$\begin{align*}
n = 29
\end{align*}$

Enrolled

$\begin{align*}
n = 3,907
\end{align*}$

- Research testing positive for bacteria ($n = 52$)
- Positive blood or CSF culture ($n = 20$)
- Enrolled multiple times during study period ($n = 22$)
- All research testing inconclusive ($n = 8$)

Excluded from statistical analysis

$\begin{align*}
n = 111
\end{align*}$

Included in statistical analysis

$\begin{align*}
n = 3,786
\end{align*}$

Positive for any virus

$\begin{align*}
n = 2,802
\end{align*}$

- Single infection with coronavirus ($n = 50$)
- Single infection with adenovirus ($n = 30$)
- Single infection with parainfluenza virus type 4 ($n = 20$)
- Other co-infections ($n = 32$)
- EVDSR Boca co-infection ($n = 1$)

Excluded

$\begin{align*}
n = 477
\end{align*}$

Negative for all viruses

$\begin{align*}
n = 584
\end{align*}$

EVDOR Single infection

$\begin{align*}
n = 38
\end{align*}$

Boca single infection

$\begin{align*}
n = 37
\end{align*}$

Boca co-infection

$\begin{align*}
n = 67
\end{align*}$

Single infection with coronavirus

$\begin{align*}
n = 2,203
\end{align*}$
Figure 2.2. Molecular detection of EV-D68 and bocavirus in pediatric patients at seven national surveillance sites
Figure 2.3. Molecular detection of bocavirus coinfections in pediatric patients with bocavirus infections at four national surveillance sites
Table 2.1. Demographic and clinical characteristics of children with EV-D68 and bocavirus single infections

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<td>N or Median % or Range</td>
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*aExcludes children with positive blood or CSF cultures*
### Table 2.2. Clinical characteristics of children EV-D68 and bocavirus infections

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<tr>
<th></th>
<th>Bocavirus single infection</th>
<th>EV/D68 single infection N=38 (1.00%)</th>
<th>Bocavirus co-infection N=37 (1.45%)</th>
<th>EV-D68 infection N=67 (2.63%)</th>
<th>Influenza N=137 (3.62%)</th>
<th>RSV N=886 (23.40%)</th>
<th>Rhino/Enterovirus N=887 (23.43%)</th>
<th>HMPV N=137 (3.62)</th>
<th>PIV 1-3 N=886 (23.40%)</th>
<th>No virus N=984 (25.99%)</th>
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<td><strong>Prior Treatment</strong></td>
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\(^a\)Bocavirus testing was conducted in California, Texas, Missouri, and Ohio

\(^b\)Excludes samples that were also positive for enterovirus D68

\(^c\)Fisher’s exact test could not converge; reported \(p\) value is from \(\chi^2\) test

\(^d\)Excludes children with positive blood or CSF cultures
References


This dissertation presents two manuscripts examining respiratory viruses in children with ARI who were hospitalized at NVSN surveillance sites. The first manuscript describes the epidemiology of established respiratory viruses (RSV, influenza, rhinovirus/enterovirus, HMPV, PIV) in an older population of pediatric patients that, to date, has not been well studied. This study identified a viral cause of illness in more than 50% of hospitalized children 5-17 years of age, which is lower than reported in children <5 years, but suggests that viruses remain an important contributor to pediatric ARI in older children. Chronic medical conditions were common in this population, occurring in ~90% of children. Distinct differences in presentation and clinical course were observed by virus detected; these were similar to presentation reported in children <5 years. Rhinovirus/enterovirus infections predominated, occurring in >35% of patients with virus-positive ARI, and, consistent with existing literature, were associated with wheezing and dyspnea. Children with influenza virus infections were less likely to have received influenza virus vaccine than children with other respiratory viruses or virus-negative ARI, which is reassuring as it suggests that influenza vaccine effectively prevents infection. In this population, cough, nasal congestion, chronic medical conditions were associated with increased odds of virus-positive ARI. Although the majority of children included in this assessment had ≥1 respiratory viruses identified, almost half did not. Additional investigation of children with virus-negative ARI is needed to better understand the causes of illness and to identify risk factors for hospitalization.
The second manuscript focuses on 2 emerging pathogens, enterovirus D68 and bocavirus. In a limited number of prior assessments, these viruses have been associated with broad spectrum of disease, including isolation from asymptomatic individuals, and have not been well described outside of geographically-isolated outbreaks. This manuscript used nationwide surveillance data to characterize EV-D68 and bocavirus infections and compared these to existing respiratory viruses. We found that, consistent with existing reports, EV-D68 was detected during spring and summer months and was associated with wheezing, cough, dyspnea, and history of asthma. Our population differed from existing reports in that children infected with EV-D68 were less likely to require mechanical ventilation and ICU admission than children with other respiratory viruses. However, our surveillance was conducted during a period of low EV-D68 circulation and did not coincide with an outbreak, which may have impacted our findings. Bocavirus was detected in a similar proportion of patients as parainfluenza virus, and most commonly occurred in combination with other respiratory viruses. In this study, children with bocavirus single infections presented similarly to children with other respiratory viruses. We observed lower respiratory tract symptoms, including wheezing, associated with bocavirus that were difficult to distinguish from RSV.

This dissertation contributes to the existing body of literature describing the epidemiology of respiratory viruses in hospitalized children. Importantly, it provides needed information about viral ARI in older children as well as emerging respiratory viruses in children of all ages.
APPENDICES

Appendix A1. UTHealth Institutional Review Board Approval

NOTICE OF PERMISSION TO UTILIZE SMART IRB RELIANCE AGREEMENT
August 15, 2018

PI: Leila Sahni
HSC-SPH-18-0686 - The epidemiology of respiratory viruses in hospitalized children and adolescents during the 2015-2016 respiratory season

CHAIRPERSON: L. Maximilian Buja, L. Maximilian Buja

PROVISIONS: This permission relates to the research to be conducted under the above referenced title. Consent must comply with the UT required In Case of Injury section and HIPAA Authorization language.

Please contact the lead study team to determine what is required by the IRB of record.

The research should not be initiated until all necessary institutional approvals and signatures have been obtained including but not limited to a fully executed clinical trial agreement and Memorial Hermann Hospital approval (if the research is being conducted at a MHH facility).
Appendix A2. Baylor College of Medicine Institutional Review Board Approval

October 18, 2018

Julie Anne Mielke, B.M.
Baylor College of Medicine
Pediatrics Academic General

H-27661 - ACTIVE SURVEILLANCE FOR ACUTE RESPIRATORY ILLNESSES AMONG PEDIATRIC PATIENTS
APPROVAL VALID FROM 8/1/2018 TO 7/31/2019

Dear Dr. BCM,

The Institutional Review Board for Human Subject Research for Baylor College of Medicine and Affiliated Hospitals (BCM IRB) is pleased to inform you that the research protocol and consent form(s) named above were reviewed and approved by Expedited procedures on 9/1/2018 by Board 6.

The study may not continue after the approval period without additional IRB review and approval for continuation. You will receive an email renewal reminder notice prior to study expiration; however, it is your responsibility to assure that this study is not continued beyond the expiration date.

Please be aware that only IRB-approved informed consent forms may be used when written informed consent is required. Any changes in study or informed consent procedures must receive review and approval prior to implementation unless the change is necessary for the safety of subjects. In addition, you must inform the IRB of adverse events encountered during the study or of any new and significant information that may impact a research participant's safety or willingness to continue in your study.

The BCM IRB is organized, operates, and is regulated with the United States Office for Human Research Protections according to the regulations codified in the United States Code of Federal Regulations at 45 CFR 46 and 21 CFR 56. The BCM IRB operates under the BCM Federal Wide Assurance No. 0000356, as well as those of hospitals and institutions affiliated with the College.

Sincerely yours,

Bambi Jo Greley, B.S.
Institutional Review Board for Baylor College of Medicine and Affiliated Hospitals
REFERENCES


57. Dare RK, Fry AM, Chittaganpitch M, Sawanpanyalert P, Olsen SJ, Erdman DD. Human coronavirus infections in rural Thailand: a comprehensive study using real-time

