Enterovirus D68
A Focused Review and Clinical Highlights from the 2014 U.S. Outbreak

Christopher M. Oermann1, Jennifer E. Schuster1, Gregory P. Conners1, Jason G. Newland1, Rangaraj Selvarangan2, and Mary Anne Jackson1

Departments of 1Pediatrics and 2Pathology and Laboratory Medicine, Children’s Mercy–Kansas City, Kansas City, Missouri

Abstract

Enterovirus D68 (EV-D68), a member of the Picornaviridae family, was first identified in 1962 and is part of a group of small, nonenveloped RNA viruses. As a family, these viruses are among the most common causes of disease among humans. However, outbreaks of disease attributable to EV-D68 have been rarely reported in the previous 4 decades. Reports from a few localized outbreaks since 2008 describe severe lower respiratory tract infection in children. In the late summer of 2014, EV-D68 caused a geographically widespread outbreak of respiratory disease of unprecedented magnitude in the United States. The Centers for Disease Control and Prevention was first notified of increased respiratory viral activity by Children’s Mercy Hospitals (CMH) in Kansas City, Missouri, and EV-D68 was identified in 50% of nasopharyngeal specimens initially tested. Between mid-August and December 18, 2014, confirmed cases of lower respiratory tract infection caused by EV-D68 were reported in 1,152 people in 49 states and the District of Columbia. A focused review of EV-D68 respiratory disease and clinical highlights from the 2014 U.S. outbreak are presented here.

Keywords: enterovirus D68; bronchiolitis; pneumonitis; epidemiology; management

Enterovirus D68

Enteroviruses are members of the Picornaviridae family, a group of small, nonenveloped, positive-sense, single-stranded RNA viruses enclosed in an icosahedral capsid (1). They are among the most common viral pathogens in humans, and are associated with a broad spectrum of clinical infections ranging from mild, self-limited illness to severe, life-threatening disease. Manifestations range from nonspecific febrile illnesses (the most common presentation), to respiratory, skin, neurologic, gastrointestinal, genitourinary, ophthalmic, cardiac, and musculoskeletal disease (2). Newborns are particularly susceptible to severe infections, including neonatal enterovirus sepsis, meningoencephalitis, and myocarditis.

Up to 15 million symptomatic enterovirus infections occur each year in the United States (3). The taxonomy of the enterovirus genus has changed from traditional groups, including polioviruses, coxsackie A and B viruses, echoviruses, and numbered enteroviruses, to now include four human species, Enterovirus A, B, C, and D, with variable numbers of serotypes within each species (Table 1). The International Committee on the Taxonomy of Viruses additionally recognizes five animal species (Enterovirus E, F, G, H, and J) and three rhinovirus species (Rhinovirus A, B, and C) within the genus.

The National Enterovirus Surveillance System, in collaboration with the Centers for Disease Control and Prevention (CDC), has monitored trends in circulating enteroviruses since 1961 (4). Between 1970 and 2005, predominant enterovirus serotypes, the prevalence ranking of individual serotypes, and the circulation patterns of individual serotypes varied. However, serotype-specific epidemic or endemic patterns remained consistent over the surveillance period. Marked seasonal variability was detected, with a late summer to fall predominance, and a peak incidence in August. A total of 44.2% of reported infections occurred in children aged less than 1 year, 15.0% in children aged 1–4 years, 11.6% in children aged 5–9 years, 11.9% in children aged 10–19 years, and 17.3% in adults. Enterovirus D68 (EV-D68; species Enterovirus D, genus Enterovirus, family Picornaviridae, order Picornavirales) was one of the most rarely reported serotypes, ranking as the 47th most common among the 58 serotypes reported.
during the 36-year surveillance period; 26 total isolates accounted for 0.1% of all enterovirus serotypes detected.

**Epidemiology**

Humans are the only reservoir for Enterovirus species A–D. Unlike most other enteroviruses, which are predominantly spread via the fecal–oral route, EV-D68 is spread through contact with infected respiratory secretions (2). Contaminated environmental surfaces may harbor active virus for prolonged periods, allowing widespread transmission. The incubation period for enteroviruses is generally 3–6 days, with viral shedding for a few days before the development of symptoms, and continued shedding in respiratory secretions for up to 3 weeks (2).

EV-D68 was first reported by Schieble and colleagues (5) in 1967 following isolation of the virus from four California children diagnosed with pneumonia and bronchiolitis in 1962. Only 26 cases of confirmed EV-D68 disease were reported between 1970 and 2005. The majority of cases (75%) was in children (4); 50% of cases were reported in the typical summer–fall season. EV-D68 was detected in respiratory specimens in all but one case, an adult with acute flaccid paralysis, in whom the virus was detected in cerebrospinal fluid (CSF). Sporadic, small, and geographically limited outbreaks of EV-D68 respiratory disease were reported globally between 2008 and 2010 (6). These included 21 patients from the Philippines, 11 patients from Japan, 24 patients from The Netherlands, and 39 patients from the United States (Georgia, Pennsylvania, and Arizona).

Subsequent reports have documented outbreaks of respiratory disease caused by EV-D68 in Great Britain, Italy, France, Thailand, China, New Zealand, and several African countries (7–15). These cohorts further characterized the seasonality of and population susceptible to EV-D68 infection. As in earlier surveillance reports, children represent the majority of cases. A summer–fall outbreak of EV-D68 respiratory disease was also reported in the United States (2012-2013). The majority of these cases were reported in the Midwest, particularly in Indiana and Ohio.

**Table 2. EV-D68 implications for clinical care**

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<th>Epidemiology</th>
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*Definition of abbreviations: CDC = Centers for Disease Control and Prevention; EV-D68 = enterovirus D68; PCR = polymerase chain reaction.*
seasonality remains consistent, though EV-D68 peaks often occur later than typical enterovirus activity. The 2014 U.S. Outbreak represents the largest and most widespread EV-D68 outbreak recognized to date, and was associated with significant morbidity and mortality. This outbreak and data from other published reports suggest an increasing incidence of EV-D68 infection, which may be caused by enhanced virulence and result in significantly increased severity of illness.

Pathogenesis

EV-D68 is an atypical enterovirus insofar as it shares certain biological features with rhinoviruses. Although most enteroviruses grow well at 37°C and under acidic conditions (pH 3.0), EV-D68 grows optimally at 33°C and does not tolerate an acidic environment (16). This likely explains the predilection of EV-D68 for the respiratory tract rather than the gastrointestinal tract, with resultant respiratory infection and spread, features characteristic of rhinoviruses rather than other enteroviruses.

Viruses enter the upper respiratory tract through direct inhalation of aerosolized particles or transmission of contaminated material to the upper respiratory tract via manual transfer of infected material from environmental surfaces. Once in the upper respiratory tract, viruses attach to the epithelial cells of the mucosal surfaces and spread locally. Attachment is thought to occur through interactions between enterovirus capsid components encoded by the VP1, 2, and 3 amino acid sequences and epithelial cell surface sialic acids, specifically N-acetylneuraminic acid α2,6-galactose (17). Additional data suggest that similarities with rhinoviruses may explain limited systemic disease, and that a predominance of N-acetylneuraminic acid α2,6-galactose receptors in the lower respiratory tract limit spread (18, 19).

Recent reports have suggested possible mechanisms for the apparent increase in EV-D68 prevalence and virulence. Tokarz and colleagues (20) conducted phylogenetic analysis of EV-D68 specimens obtained over 2 decades. Their analysis demonstrated that multiple distinct viral clades, genetically similar groups that share a common origin, have emerged and undergone rapid, worldwide spread. The distinct viral clades are thought to have originated from genetic rearrangement and diversification, leading to the emergence of clades A and C in the mid-1990s. Clade C-type underwent additional genetic alteration, leading to changes in the RNA region associated with translation efficiency, thus increasing virulence (21). Continued genetic rearrangement led to the emergence of clade B and enhanced viral persistence (15). This is supported by the demonstration of shifting antigenicity and preferential binding of EV-D68 to the α2–6-linked sialic acids found in respiratory epithelium (17). Moreover, there is speculation that these genetic mutations have led to altered antigenicity and the loss of the virus-neutralizing ability of pre-existing antibodies (10, 15, 22). Lastly, the detection of clades A, B, and C, which are genetically similar to those found worldwide, in Kenya suggests widespread transmissibility (13).

Clinical Manifestations and Diagnostic Testing

The clinical manifestations of EV-D68 have been widely reported. The respiratory tract, both upper and lower, is the primary site of disease (4–6, 9, 11, 14, 15, 20, 21, 23–27). Extremely rare reports of central nervous system disease exist (4, 28, 29). The most comprehensive descriptions of the respiratory manifestations of EV-D68 infection come from The Netherlands, two exclusively pediatric cohorts (one from the Philippines and the other from Native American children in Arizona), and CDC surveillance data.

Both reports from The Netherlands suggest a broad age range (1 mo to >80 yr) with no sex predilection (15, 26). The predominant symptoms were cough and dyspnea without systemic manifestations. Severity of illness varied greatly. The majority of patients had mild disease; however, some patients had severe disease requiring intensive care and ventilatory support. Most patients had no underlying cardiopulmonary morbidity. Imamura and colleagues (23) provided descriptive epidemiology for 21 children in the Philippines. In addition to cough and dyspnea, they identified wheeze and retractions as common findings among children. The mortality rate in this population was almost 10%. During the Arizona outbreak, which ran from mid-August to mid-September, children with EV-D68 infections were afebrile and lacked systemic symptoms, such as myalgia, fatigue, headache, or gastrointestinal abnormalities (25). Among the patients who presented with wheeze, 60% did not have a history of asthma or prior wheezing with respiratory illness. Lastly, abnormal blood counts (80%) with a banded neutrophil predominance (50%), and chest radiographs demonstrating radiographic infiltrates (56%), were common. The CDC summaries report data from outbreaks between 2008 and 2010, including some of the outbreaks described previously here, and the 2014 U.S. outbreak. Data from these summaries are consistent with those from the reports previously discussed, and indicate that EV-D68 may be a cause of severe upper and lower respiratory tract disease (6, 27).

EV-D68 has been recovered from the CSF of two patients with central nervous system disease (acute flaccid paralysis and fatal meningomyeloencephalitis) (4, 28). In addition, EV-D68 has been identified in upper respiratory specimens, but not CSF, from clusters of children with neurologic symptoms, such as acute flaccid paralysis, in California and Colorado (29, 30). Because EV-D68 was not recovered from the CSF of patients, a direct causal relationship cannot be assumed. In addition, reports of an atypical neurologic illness surfaced several weeks into the 2014 U.S. outbreak. The clinical presentation was characterized by acute onset of limb weakness with magnetic resonance imaging changes that were specific to gray matter and reminiscent of those seen with classic poliomyelitis. To date, 94 children with this presentation and with onset since August 1, 2014 have been confirmed. Extensive studies designed to detect neuroinvasive pathogens that are known to be associated with acute flaccid paralysis (West Nile virus, EV-71, and poliomyelitis), as well as EV-D68, have been negative. Based on these data, it is plausible that EV-D68 is associated with central nervous system disease, although the mechanism remains unclear.

Isolation of enteroviruses through inoculation of in vitro cell culture systems or suckling mice was the primary method for detection of enteroviruses before 1990.
In recent decades, the development of pan-enterovirus polymerase chain reaction (PCR) assays has replaced viral culture methods as the gold standard for enteroviral detection in clinical specimens (31). More recently, the introduction of rapid, multiplex, PCR-based assays for respiratory specimens allows clinical laboratories to test for multiple respiratory pathogens simultaneously. These tests are faster and more sensitive than traditional viral culture methods (32). Due to the genetic similarity between rhinoviruses and enteroviruses, the targeted region used by many multiplex PCR platforms cannot distinguish between the two species (33). Specific enterovirus serotypes are identified by sequencing the VP1 gene; the initial specimens sent to the CDC from Kansas City and Chicago during the 2014 outbreak were identified in this manner (27, 34). Since the onset of the outbreak, the CDC has designed a new real-time reverse transcriptase PCR test to identify EV-D68 without sequencing. The test is specific for EV-D68 and allows for more rapid identification.

**Summer–Fall 2014 Outbreak**

A clinical concern from a pediatric emergency medicine physician on August 15, 2014 alerted pediatric infectious disease specialists at Children’s Mercy Hospitals (CMH) to an unusual increase in the number of patients presenting to the Emergency Department with respiratory distress and severe bronchospasm. Many of these children needed hospital admission. Most admitted children required significant levels of respiratory support, including continuous nebulized albuterol, parenteral therapy for bronchospasm (magnesium and aminophylline), and noninvasive ventilation. A minority of patients required intensive care.

Review of reports monitoring viral activity confirmed an unexpectedly high number of multiplex respiratory panel PCR (Biofire) detections of rhinovirus/enterovirus during this same time frame. The level of activity was nearly four times higher than the detections from similar periods in the prior 2 years. EV-D68 infection was suspected, and consultation with CDC was obtained. A case definition was developed to facilitate specific testing in collaboration with CDC and implementation of appropriate isolation precautions. Criteria included any patient admitted to CMH with cough, respiratory distress with or without fever, or wheezing, and requiring supplemental oxygen or continuous albuterol.

Because the multiplex PCR used does not discriminate between the many different types of rhinoviruses and enteroviruses, nasopharyngeal specimens from 20 patients who presented with severe bronchospasm plus specimens from two other patients, all of whom were hospitalized in the intensive care unit at CMH, were sent to the CDC for specific viral identification. Sequencing identified EV-D68 in 19/22 (86%) specimens, including 18/19 (95%) children with severe bronchospasm. The CDC also confirmed that a physician from a geographically distinct location, Comer Children’s Hospital in Chicago, Illinois, had noted similar disease during the same time period. A total of 11 out of 14 (79%) specimens sent from those patients were identified as enterovirus D-68. Subsequent complete genomic sequencing of one specimen and partial sequencing of six additional specimens obtained during the 2014 outbreak were confirmed by the CDC to represent the three known strains of EV-D68: clades A, B, and C. They appear to be genetically related to strains detected in prior years in the United States, Europe, and Asia.

During the peak of the outbreak, Emergency Department and Urgent Care Center activity at CMH increased 15–20%, hospital inpatient census rose by 25% (300 versus 250), and intensive care unit

![Figure 1](image.png)

**Figure 1.** Rates of rapid viral panel testing and rhino-/enterovirus (RhinEnt) positivity at Children’s Mercy Hospitals (all patients). RP = rapid viral panels tested.
admissions due to respiratory infection increased significantly. At CMH, the outbreak peaked approximately 4 weeks after the putative onset in mid-August. By Week 6, inpatient admissions had returned to near-normal levels, although Emergency Department and Urgent Care Center activity remained elevated. Subsequent CDC serotyping identified EV-D68 in 48/74 (65%) admitted patients. The full extent of the disease burden is still under investigation, but from August 15 through September 16, almost 700 children, ranging in age from 1 week to 18 years, had positive testing for rhinovirus/enterovirus at CMH (Figure 1). A retrospective chart review protocol was approved by the CMH Institutional Review Board to allow more comprehensive characterization of the clinical manifestations of infection and therapeutic interventions provided. Data extraction, including all patients identified as being infected with rhinovirus/enterovirus during the outbreak, has begun.

The initial experience with the EV-D68 outbreak in Kansas City and Chicago has been recently reported (27). Children with confirmed EV-D68 during the original testing period, ranged in age from 6 weeks to 16 years. Most were school aged, many had a history of asthma, but one-third had no prior history of wheeze. In both Kansas City and Chicago, disease was recognized in outpatient and inpatient settings, there were high rates of hospital admission, and intensive care was necessary for many children.

Clinical manifestations of disease included marked tachypnea, increased work of breathing, hypoxemia, wheeze, and chest pain. Fever was present in a minority of patients. In the initial cohort of 19 Kansas City children identified by the CDC to have EV-D68, only 26% were febrile (27); 68% of children had a history of asthma or prior wheezy illness. Chest radiographs were largely normal, although some demonstrated hyperinflation or perihilar infiltrates.

Therapies used included supplemental oxygen, inhaled bronchodilators, systemic corticosteroids, and intravenous magnesium. Parenteral aminophylline and injected epinephrine were used less commonly. Unlike most children with viral lower respiratory tract infections (LRTIs), patients treated for respiratory infection during this outbreak appeared to respond to the combination of inhaled bronchodilators and systemic corticosteroids, albeit more slowly than is typically expected with asthma triggered by LRTI. Supportive care included high-flow nasal cannula oxygen, bilevel positive airway pressure, and rarely, intubation with mechanical ventilation; one patient in Chicago required extracorporeal membrane oxygenation. Although no patient deaths directly attributed to EV-D68 were confirmed at CMH, nationally, there have been reports of 12 deaths in children confirmed to have EV-D68 infection during the 2014 U.S. Outbreak. Investigation as to the role of EV-D68 in these deaths is ongoing.

As of 18 December, 2014, CDC reports that 1,152 patients from 49 states and the District of Columbia have been confirmed to be EV-D68 positive. Testing has occurred at the national, state, and hospital level. Nationally, CDC has tested more than 2,000 specimens, with approximately 40% positive for EV-D68. This is undoubtedly an underestimate of the true burden of disease, as many children with mild respiratory disease were not tested.

Managing the Illness

At the time of the outbreak, there was a paucity of data regarding the medical management of patients with respiratory infection caused by EV D-68. Jacobson and colleagues (25) provided information on the treatment of the children involved in an Arizona outbreak. Among their patients, 83% required supplemental oxygen, 83% were treated with albuterol, 61% received antibiotics, and 56% received systemic corticosteroids. The median length of hospitalization was 1.5 days. Similarly, care at CMH was supportive and followed basic guidelines for treating children with asthma with severe bronchospasm; no investigational treatments were undertaken. In contrast to the Arizona experience, few patients received antibiotics at our hospital, and all children with severe bronchospasm who were over 12 months of age received systemic corticosteroids.

We found that a key component of disease management related to infection prevention and control. As there are no vaccines available for EV-D68 prophylaxis, conventional infection control measures in both community and healthcare settings were used. For hospitalized patients, precautions included observation of appropriate infection control policies (standard, contact, and droplet). We recommended community practices including meticulous hand hygiene, proper cough and sneeze etiquette, avoidance of sick contacts, and environmental decontamination of potentially infected surfaces and objects, particularly in settings with large congregations of children. In addition, we recommended that patients with underlying cardiorespiratory morbidity keep in close contact with their healthcare providers and adhere to existing care plans, including asthma action plans.

No specific antiviral therapy is available to treat EV-D68 infection. Drugs, such as pleconaril, pocapavir, and vapendavir, demonstrate variable activity against some enteroviruses and are under development, but are not commercially available. Pleconaril prevents viral uncoating and attachment to host epithelial cells (35). Pocapavir works in a similar way, preventing viral uncoating and RNA release, and has been developed primarily as a potential therapy for polio, but shows broad spectrum antienterovirus activity (36). Another capsid inhibitor, vapendavir, has been developed primarily as a potential treatment for rhinovirus, but has shown activity against other enteroviruses (37). “CDC has tested these drugs for activity against currently circulating strains of EV-D68, and none of them has activity against EV-D68 at clinically relevant concentrations” (38).

Lessons Learned

EV-D68 appears to be increasing in prevalence and virulence. The reasons for this are not well understood, but may relate to viral diversification and genetic rearrangement. Current PCR platforms are not specific, and will identify EV-D68 as rhinovirus/enterovirus. Specific identification requires referral to CDC. EV-D68 infection can be associated with severe upper and lower respiratory tract disease. In addition to supportive care, and unlike most viral LRTIs, patients with EV-D68 infection benefited from the use of bronchodilators and systemic steroids. Prolonged therapy was often necessary. Routine community and healthcare facility infection control practices were essential in preventing the spread of disease. For details, see Table 2.

Future Directions

The clinical significance of EV-D68 and its capacity for geographically widespread
outbreaks involving very large numbers of patients is only now being appreciated. CDC reports confirm a total of 1,152 people in 49 states and the District of Columbia with infection caused by EV-D68 between August and mid-December 2014. Because only the most severely ill children were tested, the full burden of disease remains unknown, and it is likely that milder disease occurred in a substantial number of children who went undiagnosed. It will be critical to continue to monitor the epidemiology of EV-D68 and understand key elements leading to its recent increased incidence and global spread. Considerable research will be needed to characterize the pathophysiologic processes involved in viral adhesion, local and systemic spread, and increased virulence. Finally, comprehensive analysis of existing data sets will be necessary to determine optimal prevention and treatment strategies, and to define strategies to evaluate the role of EV-D68 in future late summer–fall seasonal outbreaks of respiratory infection.

Author disclosures are available with the text of this article at www.atsjournals.org.

References


