

That other EVD: Enterovirus-D68 – what's it all about?

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Until recently, we suspect that very few of those reading the present article were familiar with the D68 serotype of enterovirus. However, starting in August 2014, reports arose of severe respiratory disease in children with enterovirus D68 (EV-D68) in both Chicago, Illinois, and Kansas City, Kansas (USA) (Table 1) (1), right in the midst of concerns worldwide about a more virulent pathogen that is also abbreviated as EVD – Ebola virus disease.

In 2012, the taxonomy for the genus *Enterovirus*, belonging to the family *Picornaviridae*, was revised to include 12 species: enterovirus A through J (with no letter 'I' because it could be confused with the number one) and rhinovirus A, B and C (2). Each of the 12 species is subdivided into serotypes that are named using an abbreviation of the appropriate common virus name (with the human ones being enterovirus [EV], coxsackievirus [CV], echovirus [E], poliovirus [PV] and rhinovirus [RV]) and a number (3). Most of the human pathogens belong to *E. enterovirus* A through D. To make the nomenclature even more confusing, most commercial laboratory respiratory virus testing methods use multiplex polymerase chain reaction (PCR) assays that do not distinguish between enterovirus and rhinovirus, often resulting in the grouping of these viruses in laboratory reports.

EV-D68, belonging to *E. enterovirus* D, was first detected in 1962 in four children in California (USA) with respiratory disease (4). Since then, clusters of cases of respiratory disease linked to EV-D68 have been described worldwide (Table 1). EV-D68 was initially named human rhinovirus 87 because it has the acid lability typical of rhinovirus (5), until genetic and antigenic analysis led to it being reclassified as an enterovirus (6). Voluntary and passive enterovirus surveillance in the United States (US) from 1970 through 2005 identified EV-D68 as a rare serotype (ranked 47th of 58 serotypes identified), with one unusual feature being that only one-half of the 26 cases occurred during the typical US enteroviral season (June through October). The percentage of cases occurring among individuals ≥ 20 years of age (23.5%) was higher than for most enteroviral serotypes (7).

Despite the fact that apparent outbreaks with severe clinical manifestations have occurred on different continents for >50 years, the epidemiology of EV-D68 is far from fully elucidated. Most clinicians only obtained access to routine diagnostics for enterovirus when molecular detection methods became widely available in the past decade. As previously mentioned, laboratories commonly do not distinguish between enterovirus and rhinovirus. Even when these viruses are differentiated, enterovirus serotyping is typically only available as part of an outbreak investigation. Therefore, the incidence of EV-D68 infection, the spectrum of clinical manifestations, the ages of those with infection and disease, and both short- and long-term outcomes are unclear.

Why was there so much more 'fuss' about the 2014 EV-D68 clusters of cases compared with previous outbreaks? It appears likely that this heightened level of concern occurred because, due to more rapid availability of strain typing, the virus linked to the US clusters was identified before the outbreak had passed. Furthermore, to have simultaneous outbreaks with multiple pediatric intensive care admissions in US cities that are 700 km apart was alarming, indicating that we could be on the verge of a widespread severe outbreak.

In response to the reports of the two US outbreaks, the Provincial Laboratory for Public Health in Alberta retrospectively tested 230 nasopharyngeal specimens from children (<18 years of age) submitted from July 1, 2014 to September 10, 2014 from which enterovirus or rhinovirus had already been detected. None of the specimens from July yielded enterovirus. There were 83 enterovirus specimens from August and September, of which 49 (59%) proved to be D68. As of November 7, 2014, an additional 62 cases of EV-D68 in Alberta have been detected in children through prospective testing (8).

In the US, almost all states (47 of 50) reported cases of EV-D68 as of November 12, 2014, with a total of 1116 cases detected. EV-D68 was clearly the predominant serotype because it was detected from approximately 40% of all specimens submitted to the US Centers for Disease Control and Prevention (CDC) (Georgia, USA) for enteroviral testing, with approximately 30% being positive for other enteroviruses or rhinovirus (9). Asthma appeared to be a risk factor for the detection of EV-D68 in Alberta and in the US (8,9). This link had not been described with previous outbreaks, possibly because they were dramatically smaller. Other than children in the two index outbreaks in Chicago and Kansas City (1), it appears that most children in whom EV-D68 was detected in 2014 experienced uneventful hospital admission with respiratory tract infections. One would assume that if EV-D68 is similar to all other enteroviruses, patients in whom EV-D68 is detected represent 'the tip of the iceberg', with many others not being tested because they did not require hospital admission, did not have testing performed or did not have symptoms that would prompt testing for respiratory viruses. Anecdotally, pediatric emergency physicians in Alberta described seeing more severe cases of asthma in August and September than expected for that time of year, but it is not possible to determine whether this was related to EV-D68. Twelve deaths had been reported in the US as of November 12, 2014 (9) but the clinical details and the role that EV-D68 played in these deaths is unclear.

To further muddy the waters, on September 24, 2014, the CDC announced that nine children in Colorado (USA) had presented from August 8 to September 15, 2014 with acute flaccid paralysis (AFP), of whom four ultimately had EV-D68 detected from nasopharyngeal specimens (10). Features of these cases that were unusual for AFP included focal extremity involvement and magnetic resonance imaging changes in the anterior horn cells, specifically nonenhancing lesions on multiple levels of the spinal cord usually restricted to the gray matter (anterior myelitis). Of note, of 245 cases of AFP in California from 1992 to 1998, none had anterior myelitis documented on imaging (11). In response to the Colorado cases, the CDC began performing surveillance for cases of AFP with onset August 1, 2014 or later that had focal limb weakness and anterior myelitis on magnetic resonance imaging. As of November 12, 2014, 75 cases of AFP from 29 states had been reported through this surveillance program (12). In Canada, it became evident that most pediatric hospitals had also admitted a small number of similar AFP cases, including at least four cases in Calgary (Alberta) (13) and two in Edmonton (Alberta). As with the Colorado cases, many did not have EV-D68 detected. It is not

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TABLE 1
Details of clusters of cases of enterovirus D68, published up to October 31, 2014

Country; year (reference[s])	Months	Cases, n	Age of patients	Spectrum of illness	Severity of illness	Comments
California, United States; 1962 (4)	October–December	4	10 months to 3 years	Bronchiolitis or pneumonia	NR	
Philippines; 2008–2009 (20,21)	October–March	21	1 month to 9 years; 17 (81%) were ≤4 years of age	All respiratory	2 deaths	NPAs tested on 816 children up to 14 years of age with severe pneumonia
Georgia, United States; 2009 (21)	September–April	6	All adults of whom 3 were >50 years of age	All respiratory	Only 3 admitted to hospital, for a median of 4 days	Typing performed retrospectively due to perceived increase in severity of respiratory tract infections
Pennsylvania, United States; 2009 (21)	August–October	28	15 (54%) were ≤4 years of age	All respiratory	Median hospitalization of 5 days; 15 admitted to PICU but no deaths	Typing performed retrospectively due to doubling of number of cases with rhinovirus detected
Arizona, United States; 2009 (21)	August–September	5	1 (20%) was ≤4 years of age	Pneumonia	Median hospital stay of 1.5 days	Typing performed retrospectively due to increase in number of cases of pneumonia in a pediatric hospital
United States; 2010 (22)	NR	7	Only adults sampled	Respiratory	NR	Reanalyzed 97 throat swabs submitted on recruits at 8 military bases, 2000–2006
Japan; 2010 (21)	July–October	>120 (clinical data only available for 11)	10 (90%) ≤4 years of age	10 had respiratory illness and 1 experienced a febrile seizure	1 death (presented with cardiac arrest)	
Netherlands; 2010 (21)	August–November	24	11 (46%) were ≤4 years of age and 12 (50%) were adults	All respiratory	5 ICU admissions but no deaths	
Chicago, Illinois, United States; 2014 (1)	August–?	11	20 months to 15 years	All respiratory	10 ICU admissions of whom 2 required mechanical ventilation (1 of whom required ECMO); 2 others underwent positive pressure ventilation	8 of 11 had a history of asthma
Kansas City, Kansas, United States; 2014 (1)	August–?	19	6 weeks to 16 years	All respiratory	All 19 were admitted to PICU; 4 required positive pressure ventilation	13 of 19 had a history of asthma. Only 5 of 19 had fever

ECMO Extracorporeal membrane oxygenation; ICU Intensive care unit; NPA Nasopharyngeal aspirate; NR Not reported; PICU Pediatric ICU

clear how commonly EV-D68 is shed in stool or how long the virus persists in the nasopharynx. Other enteroviruses that cause AFP, such as poliovirus, are typically not detected in the cerebrospinal fluid (CSF). Not all commercial multiplex polymerase chain reactions have the primers required for detection of EV-D68. It is possible that AFP is a postinfectious phenomenon with EV-D68 infection. For all of these reasons, failure to detect EV-D68 in any clinical specimens from children with AFP certainly does not exclude it as a pathogen.

Are there previous cases of neurological disease from EV-D68? The first published case was in 2005 from the US – a young adult with AFP in whom EV-D68 was detected in CSF (7). This was one of the rare cases in which EV-D68 was detected in a nonrespiratory sample (7). A five-year-old boy in the US also had the virus detected in CSF when he presented with AFP and pneumonia (14). There has been a marked increase in the number of AFP cases with anterior myelitis in California starting June 2012 with 23 cases reported up to June 2014.

Two of the 23 cases had EV-D68 detected from respiratory samples (11). Many countries perform active surveillance of cases of AFP as part of the WHO polio eradication program, so it appears to be likely that more AFP cases linked to EV-D68 will begin to be reported given the current level of interest in the virus.

What does the future hold for EV-D68? Most enterovirus infections in temperate climates occur in August through October. US data showed a low or declining number of EV-D68 cases in 39 of 43 reporting states during the week of October 19 to 26, 2014 (9). Therefore, by the time that the present column is published, there will likely be no or very few new cases. The previously mentioned US surveillance system for enterovirus showed that predominant serotypes have changed over time, from 1975 to 2005 (7). Over the subsequent three years, CV-B1 became the predominant serotype (15). The major unanswered questions include: where (if anywhere) will EV-D68 become predominant next enteroviral season, or will it cause disease

outside of the classic enteroviral season? Has the virus mutated and will it continue to be associated with more severe clinical manifestations, including AFP? What morbidity is it responsible for in adults?

The antiviral drug pleconaril has activity against many enteroviruses (including RV). Licensing could presumably be expedited because large clinical trials were performed in the 1990s for children and adults with aseptic meningitis and for the common cold. However, pleconaril has been reported to not have activity against EV-D68 (16).

Other enterovirus serotypes have shown the propensity to cause severe disease. Since the late 1990s, EV-A71 has become an ongoing cause of outbreaks of hand, foot and mouth disease with severe cardio-pulmonary and neurological manifestations in children in the Asia-Pacific region (17). This had led to the development of a vaccine that appears to have favorable immunogenicity and safety in phase II trials

(18). Infections with EV-A71 occur in Canada but have not been linked to serious disease (19). A pessimistic prognostication would be that EV-D68 may become an ongoing threat in Canada the way the EV-A71 has in Asia. If the EV-D68 association with AFP proves to be causal, there could be an urgent need to develop a vaccine. It would be ironic and devastating if polio eradication is finally achieved, yet a new polio-like virus moves in to take its place.

It appears that EV-D68 is fading away, as enteroviruses are expected to do when the snow begins to fly. However, given the link with severe respiratory disease, AFP, and death, this will remain a virus of interest. Just as technology has helped to rapidly prove that an outbreak of EV-D68 is occurring, we can hope that the same technology will help us to eventually combat the virus should a link between EV-D68 and major morbidity be established.

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