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Detection of Enterovirus D68 in Samples Enrolled in the New Vaccine Surveillance Network from January to December 2022

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Abstract (Modified)

Background: In 2014, the largest cohort of pediatric EV-D68 disease in the U.S. was reported by Children's Mercy Kansas City (CM) (Midgley, et al. 2014). Shortly after, reports of increased EV-D68 around the world were announced. Since then, EV-D68 has circulated even years during the summer to fall months (Gummersheimer, et al. 2022). In June 2022, an increase in Rhinovirus/Enterovirus (RV/EV) infections with symptoms resembling EV-D68 outbreaks were noted earlier than usual in Kansas City's clinical setting. Common symptoms of EV-D68 infection resemble asthma exacerbation. Severe EV-D68 infection has been linked to those with a history of asthma. We aimed to determine the rate and epidemiology of EV-D68 infection in children enrolled in the Center of Disease Control and Prevention (CDC)'s New Vaccine Surveillance Network (NVSN) acute respiratory illness (ARI) 20222 study.

Methods: Between January-December 2022, children <18 years old seen at CM with ARI symptoms were enrolled into the CDC's NVSN ARI study. Nasal samples collected in universal transport media (UTM) were tested by NxTAG® Respiratory Pathogen Panel (Luminex) multiplex assay which can simultaneously detect 20 respiratory pathogens. Those with RV/EV detections were further tested by a real-time EV-D68 RT-PCR. Demographic and clinical information was collected throughout the season.

Results: A total of 2,312 samples were tested; RV/EV was detected in 28% (n=638). EV-D68 was detected in 15% (n=93/638) of positive RV/EV samples, with an overall 4% (n=93/2514) rate of EV-D68 detection. EV-D68 was found to be highest in Black/African American children (50%), in children ages 1-4 years (66%) and in males (57%). EV-D68 was more prevalent in subjects enrolled in the emergency department setting (57%) compared to the inpatient (30%) and clinic settings (13%). The most common symptoms reported by families of enrolled children were coughing (97%), nasal congestion (90%), and shortness of breath (77%). All 93 EV-D68 positives were detected in the summer to fall months (June to September).

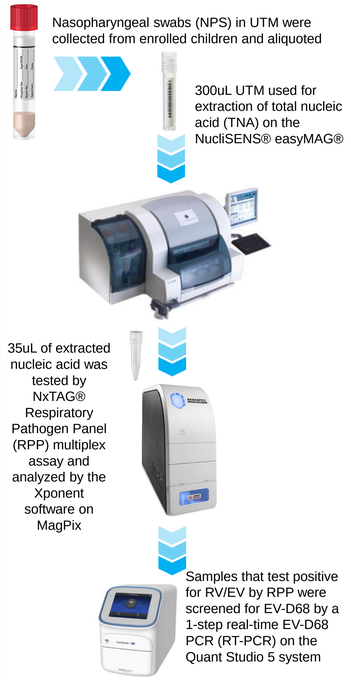
Conclusions: The detection of EV-D68 within the CDC NVSN brought an early awareness to the symptoms and diagnosis of this infection. Findings were reported to the CDC and in September 2022, a health advisory regarding the increase in EV-D68 prevalence in hospitals and clinics nationwide was reported (CDC Health Alert Network 2022). Data gathered at CM, along with that from other participating sites can contribute to early detection and preparedness for future outbreaks

Introduction

- Although rhinovirus (RV) infection is a common cause of ARI, a significant portion of ARI can also be caused by an enteroviruses (EV), including EV-D68.
- In 2014, the first large U.S. EV-D68 outbreak was reported by Children's Mercy Kansas City (CM) (Midgley, et al. 2014).
- EV-D68 can cause severe cough, difficulty breathing, and wheezing. It can exacerbate asthma symptoms necessitating intensive care. More severe cases are associated with outbreaks of acute flaccid myelitis (AFM) (Gummersheimer, et al. 2022).
- Multiplex assays currently used in clinical laboratories in the U.S. do not specifically detect EV-D68 and results are generally reported as RV/EV positive. ED-D68 is detected by research testing alone.
- EV-D68 follows a biennial pattern of circulation that has been observed with peaks in the late summer through fall months (Gummersheimer, et al. 2022).
- In June 2022, an increase in RV/EV infections with symptoms resembling EV-D68 were noted in Kansas City earlier than the usual late summer/early fall seasonality.

Materials and Methods

Figure 1: Workflow for EV-D68 detection



- Samples with CT values of 40.0 and below were reported positive for EV-D68
- Extracts are sent to CDC for further testing

Results

Table 1: Specimen results from ARI and healthy control (HC) children a) overall RV/EV detection b) CT values of EV-D68 positive samples

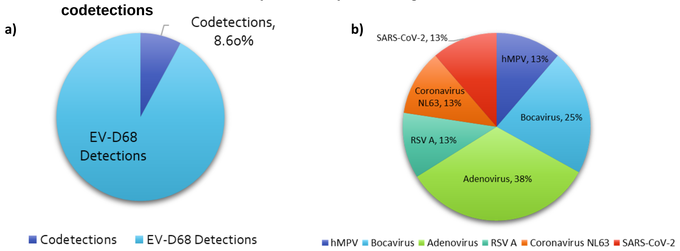
a)

Total NVSN ARI & HC Samples Tested	2312
Total RH/EN Positives	638 (27.60%)
EV-D68 Positives	93 (14.58%)
YTD EV-D68 Positives	4.02%

b)

Min CT	14.5
Q1 CT	25.04
Median CT	28.33
Q3 CT	32.57
Max CT	41.01

Figure 2: Coinfection Data for EV-D68 Positive Samples a) Percentage of codetections within EV-D68 positives b) Percentage of viruses found within the codetections

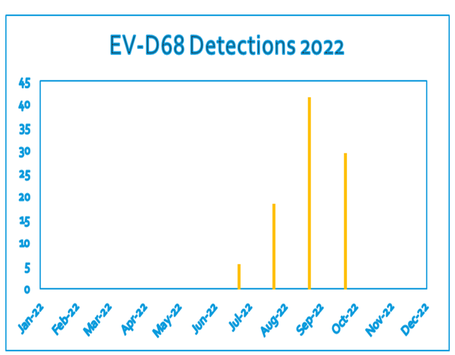


Seasonality

Table 2: Samples tested for RV/EV and EV-D68 by months during the study period

Month	RPP Tested	RV/EV Positives	RV/EV Pos %	EV-D68 Positives	EV-D68 Pos %
Jan-22	177	20	11.30%	0	0.00%
Feb-22	143	34	23.78%	0	0.00%
Mar-22	177	47	26.55%	0	0.00%
Apr-22	179	49	27.37%	0	0.00%
May-22	163	54	33.13%	0	0.00%
Jun-22	**160**	**54**	**33.75%**	**5**	**9.26%**
Jul-22	**124**	**41**	**33.06%**	**18**	**43.90%**
Aug-22	**177**	**70**	**39.55%**	**41**	**58.57%**
Sep-22	**244**	**99**	**40.57%**	**29**	**29.29%**
Oct-22	301	78	25.91%	0	0.00%
Nov-22	297	69	23.23%	0	0.00%
Dec-22	170	23	13.53%	0	0.00%
Total	2312	638	27.60%	93	14.58%

Figure 3: Positive EV-D68 detections



Demographic Data

Figure 4: EV-D68 Positive Samples by Age in Years

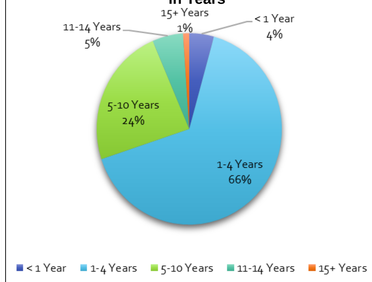


Figure 5: EV-D68 Positive Samples by Gender

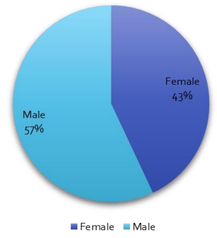


Figure 6: EV-D68 Positive Samples by Race

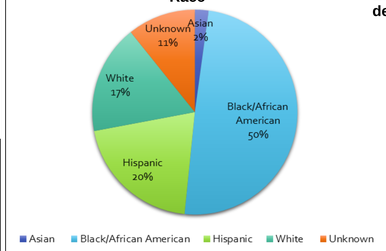
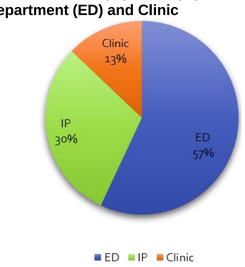


Figure 7: EV-D68 Positive Samples by Enrollment Site (Inpatient (IP), Emergency department (ED) and Clinic



Clinical Data

Table 3: Symptoms reported in EV-D68 positive children with frequency of symptoms in descending order

Symptoms	Number Reported Yes	Percentage
Cough	**90**	**96.77%**
Nasal Congestion	**84**	**90.32%**
Shortness of Breath	**72**	**77.42%**
Wheezing	**61**	**65.59%**
Irritability	**57**	**61.29%**
Fever	54	58.06%
Loss of Appetite	54	58.06%
Fatigue	35	37.63%
Sore Throat	30	32.26%
Red/Pink eye(s)	20	21.51%
Vomiting	15	16.13%
Earache	15	16.13%
Abdominal Pain	15	16.13%
Headache	15	16.13%
Diarrhea	14	15.05%
Skin Rash	12	12.90%
Chills	11	11.83%
Asthma	11	11.83%
Muscle Aches	8	8.60%
Airway Disorders	2	2.15%
Confusion	1	1.08%
Use of Supplemental Oxygen	1	1.08%
Chronic Lung Condition	1	1.08%
Seizures	0	0.00%

Discussion

- Of the 2,312 samples tested by RPP, 27.60% were RV/EV positive. EV-D68 was detected in 14.58% of the RV/EV positives with an overall EV-D68 detection rate of 4.02% from January to December 2022.
- The average cycle threshold (CT) for EV-D68 this season was 28.69.
- Of the 93 EV-D68 positives this year, 8 (8.60%) of the samples were detected as coinfections. The most frequent codetection was Adenovirus (38%).
- Within the EV-D68 season of 2022, 705 samples were tested by RPP, 37.45% were RV/EV positive. The detection rate of EV-D68 between the months of June to September 2022 was 35.23%.
- All 93 positive EV-D68 samples for 2022 were detected in the early summer to fall months, rather than its typical pattern of late summer to fall.
- EV-D68 was detected to be highest in Black/African American children (50%), in children ages 1-4 years (66%) and in males (57%).
- EV-D68 was more prevalent in subjects enrolled in the emergency department setting (57%) compared to the inpatient (30%) and clinic settings (13%).
- Of the 24 symptoms screened for, the most common symptoms in children with EV-D68 were cough (97%), nasal congestion (90%), and rapid shallow breathing (77%).

Conclusion

- EV-D68 was detected within the Kansas City NVSN samples in higher numbers during concurrent routine surveillance testing. These findings were reported to the CDC and positive extracts were sent to CDC throughout the season.
- Unusually high numbers of RV/EV infections were noted clinically in early summer of 2022. After the EV-D68 detections within the Kansas City NVSN study were found, clinical samples were pulled and tested for EV-D68 in order to get more data on the outbreak within Kansas City.
- In early September 2022, the CDC released a health advisory to report increased EV-D68 prevalence in hospitals and clinics nationwide.
- Data gathered during active surveillance at CM, and at other NVSN sites can contribute to early infectious disease detection and preparedness for future outbreaks.

References

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