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Relation between enteroviruses and acute flaccid paralysis

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Abstract:
Acute flaccid myelitis (AFM) is a rare disease that affects the spinal cord,and its by several agent like Guillain-Barre Syndrome , poliovirus, non-polio enterovirus**(most common EV-D68).t**he onset of AFM is sudden.Symptoms include weakness in one or more limbs (arms, legs), Flabby muscles affecting the face.**Enterovirus D68** is a single-stranded positive-sense RNA virus,was previously known as **rhinovirus 87** until it was re-classified in 2002,It likely spreads from person to person.the aim of study is to know the is to know the relation of EV-D68 to the of acute flaccid paralysis.the study conducted a retrospective case–control study of children who had received medical care for any illness necessitating collection of nasopharyngeal specimens for respiratory pathogen testing in Colorado during August 3, 2014–October 18, 2014. the result show that **EV-D68** is the most common cause.

Introduction:

Acute flaccid myelitis (AFM) is a rare disease that affects the spinal cord, the part of the nervous system that carries messages to and from the brain. Before being described in 2014, AFM might have been diagnosed as a type of transverse myelitis**.** However, one difference between AFM and transverse myelitis has been found by magnetic resonance imaging (MRI) scans. The gray matter of the spinal cord is inflamed in people with AFM. AFP may be caused by several agents including Guillain-Barre Syndrome (GBS) (the most common cause), poliovirus, non-polio enterovirus**(most common EV-D68)**, adenovirus, acute West Nile virus, campylobacter spp, transverse myelitis, peripheral neuropathy, acute non-bacterial meningitis, tick paralysis, and brain abscess. The onset of AFM is sudden (acute). Symptoms include weakness in one or more limbs (arms, legs), Flabby muscles affecting the face, head and neck, which might cause one side of the face to fall lower than the other, problems with swallowing or talking, problems with swallowing or talking, Weakness in muscles and nerves in the respiratory system, Pain in an arm or leg, and Inability to urinate. **Guillain-Barre Syndrome** is a reactive self-limited auto-immune disease in which the body's immune system attacks part of the peripheral nervous system and which presents as an acute generalized weakness. **Enterovirus D68** is a single-stranded positive-sense RNA virus of the *Picornaviridae* family, belonging to the species enterovirus D. was first isolated from respiratory samples in 1962 in California, United States, was previously known as **rhinovirus 87** until it was re-classified in 2002, and can cause mild to severe respiratory illness, or no symptoms at all. Mild symptoms may include runny nose, sneezing, cough, body aches, and muscle aches, and Severe symptoms may include wheezing and difficulty breathing. It likely spreads from person to person when an infected person coughs, sneezes, or touches a surface that is then touched by others.

Aim of study:

The of aim of study is to know the relation of EV-D68 to the of acute flaccid paralysis in the last years.

Materials and methods:

Was conducted a retrospective case–control study of children who had received medical care for any illness necessitating collection of nasopharyngeal specimens for respiratory pathogen testing in Colorado during August 3, 2014–October 18, 2014 (the epidemiologic weeks when confirmed AFM cases were identified). AFM case-patients were defined as children <21 years of age who had acute neurologic illness characterized by focal weakness of >1 limbs, magnetic resonance imaging (MRI) findings of spinal cord lesions largely restricted to gray matter, and no identified etiology, per CDC case definition (6,7,9,10).

We also identified 2 control groups of children for whom nasopharyngeal specimens had been obtained while they were CHCO outpatients during the study period. We selected outpatients as controls because most AFM case-patients had respiratory signs and symptoms and were evaluated as outpatients before neurologic symptoms developed. The first control group (respiratory pathogen panel [RPP]–tested controls) included children who were evaluated as outpatients and for whom nasopharyngeal specimens had been tested by multiplex RPP PCR (FilmArray; BioFire Diagnostics LLC, Salt Lake City, UT, USA), which detects adenovirus; coronaviruses HKU1, NL63, 229E, OC43; influenza viruses A(H1N1)pdm09, A(H3), and B; metapneumovirus, parainfluenza viruses 1–4; respiratory syncytial viruses A and B; enterovirus/rhinovirus; Bordetella pertussis; Chlamydophila pneumoniae; and Mycoplasma pneumoniae. The second control group (B. pertussis [BP]–tested controls) included children who were evaluated as outpatients and who had nasopharyngeal specimens obtained for PCR testing for B. pertussis. We excluded from the study infants <12 months of age and children >18

years of age because AFM patients in these age groups had not been identified in Colorado during this period. If multiple specimens from the same child were submitted for testing from different times during the study, only the first was included. Specimens submitted for RPP and BP testing from the same child and on the same date were considered for the RPP analysis only.

Was also analyzed results of FilmArray testing for all patients admitted to the pediatric intensive care unit (PICU) at CHCO during July–November 2014. We chose this population because all patients admitted with respiratory symptoms routinely undergo FilmArray testing and results would provide a representative view of pathogens circulating in the community and resulting in severe respiratory illness at the time of the outbreak.

Result:

The peak of AFM diagnoses coincided with the peak of EV-D68 respiratory infection detections at CHCO (6,7). Viral analyses of the specimens from PICU patients indicated that the predominant virus causing severe respiratory illness during the outbreak was EV-D68, followed by enterovirus/rhinovirus species excluding EV-D68 (Figure 1). Of the 203 specimens from PICU patients that were positive by RPP and sent to CDC for further testing, 100 (49%) were positive for EV-D68 (Figure 2). Among the other enterovirus/rhinovirus species, no predominant virus was in circulation. These species included human rhinoviruses, echoviruses, and coxsackieviruses A and B; some specimens could not be typed.Overall, during the outbreak period in Colorado, we identified 13 patients who had acute neurologic disease with limb weakness, cranial nerve dysfunction, or both. Use of the CDC AFM case definition resulted in exclusion of 2 patients with acute neurologic disease who

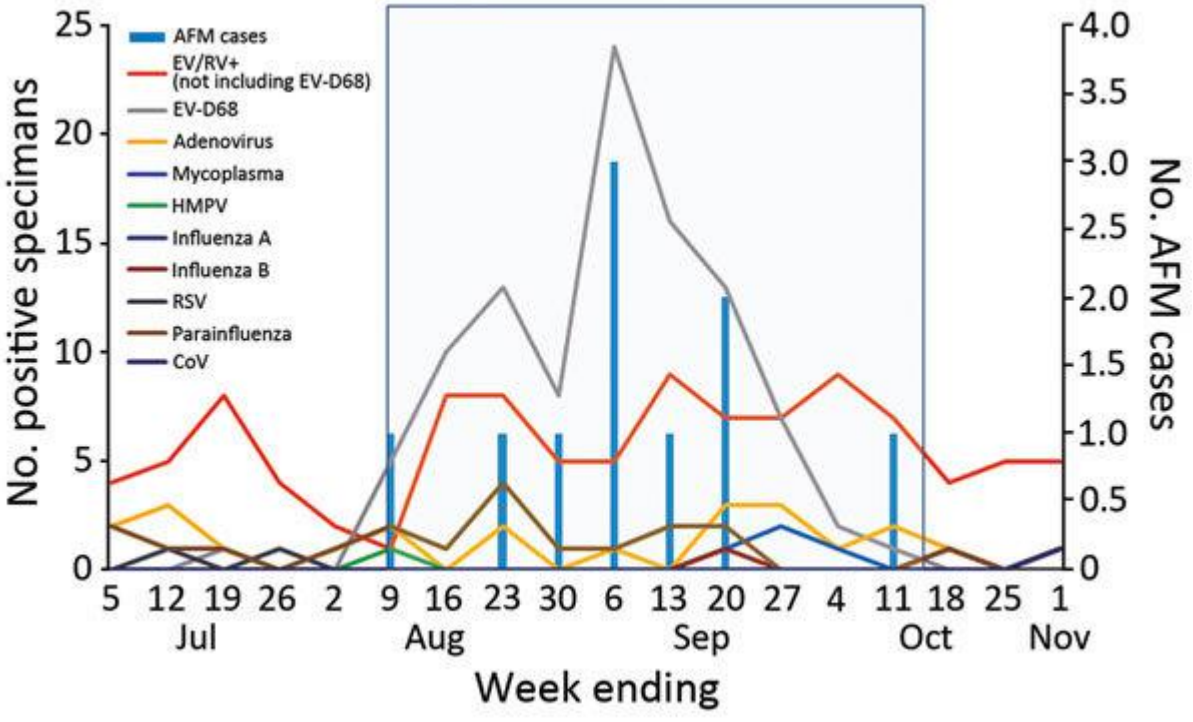

Figure 1

).Table 1

Characteristics of patients with acute flaccid myelitis and control patients, Colorado, August 3–October 18, 2014*

Characteristic	Case-patients, n = 11	Control patients: RPP-tested†	p value	Control patients: BP-tested‡	p value
Sex, no. (%)					

Characteristic	Case-patients, n = 11	Control patients			
		RPP-tested†	p value	BP-tested‡	p value
M	8 (73)	62 (59)	0.52	124 (53)	0.24
F	3 (27)	43 (41)	NA	108 (47)	NA
Age, y, median (IQR, range)	8 (9, 1–18)	5 (8, 1–18)	0.05	7 (8, 1–18)	0.14
Respiratory symptoms, no. (%)	10 (91)	82 (79)§	0.69	221 (97)¶	0.25
Fever, no. (%)	10 (91)	79 (76)§	0.45	73 (32)#	<0.001
Hospitalized for respiratory symptoms, no. (%)	0	11 (11)**	0.60	12 (5)¶	1.00
Enterovirus testing, no. (%)					
EV/RV negative	4 (36)	85 (81)	NA	165 (71)	NA
EV/RV positive, excluding EV-D68	3 (27)	14 (13)	0.08††	36 (16)	0.12††
EV-D68 positive	4 (36)	6 (6)	0.02‡‡	31 (13)	0.03‡‡
Type of specimen, no. (%)					
Nasopharyngeal swab	6 (55)	57 (54)	1.00	192 (83)	0.04
Nasopharyngeal aspirate/wash	5 (45)	48 (46)	NA	40 (17)	NA
Time to specimen collection, d, median (IQR, range)§§	10 (7, 7–36)	5 (5, 0–31)¶¶	<0.001	14 (14, 1–120)##	0.91
Epidemiologic wk of specimen collection, median (range)	37 (33–42)	38 (32–42)	0.58	(32–42)	0.89

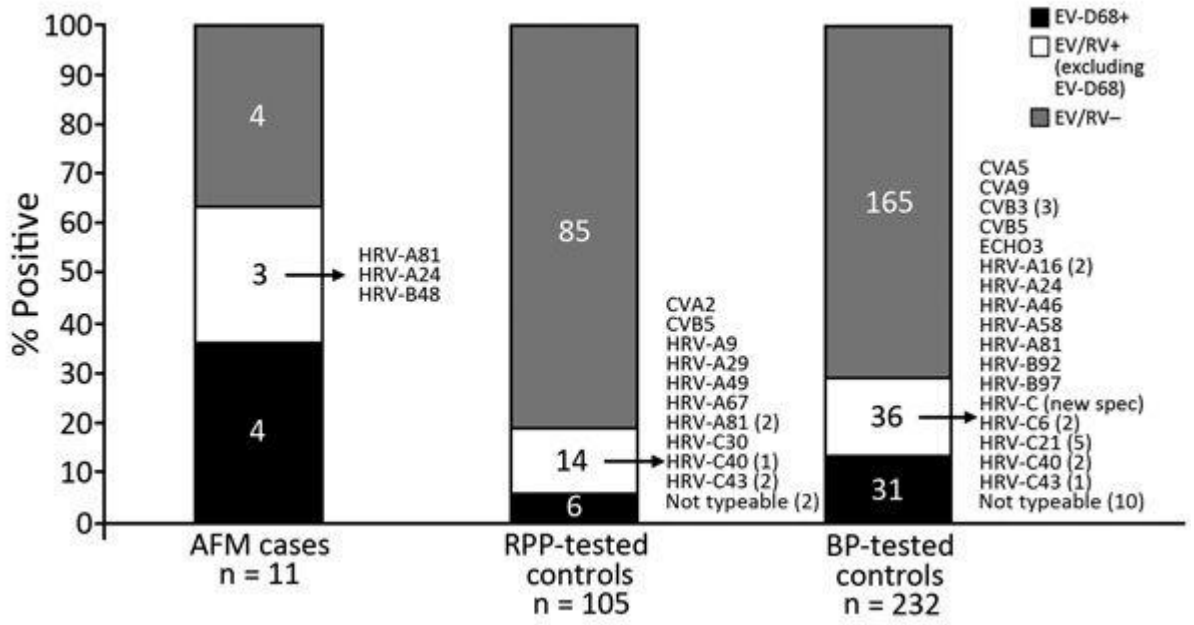

Figure 2

Comparing AFM case-patients with RPP-tested controls, we found that AFM case-patients were older (median age 8 years vs. 5 years, respectively; p = 0.05) and that specimens from AFM case-patients were collected later than specimens from RPP-tested controls (median 10 vs. 5 days after respiratory symptom onset, respectively; p<0.001). We found no statistically significant differences between these 2 groups with regard to sex, presence of upper or lower respiratory symptoms, presence of fever, hospitalizations for respiratory symptoms, type of specimen obtained, or epidemiologic week of specimen collection.

Comparing AFM case-patients with BP-tested controls, we found that AFM case-patients were more often febrile (91% vs. 32%, respectively; p<0.001) and had fewer nasopharyngeal specimens collected by swab than by aspiration (55% vs. 83%, respectively; p = 0.04). We found no statistically significant differences between these 2 groups with regard to sex, age, presence of respiratory symptoms, hospitalizations for respiratory symptoms, timing of specimen collection, and epidemiologic week of specimen collection. Furthermore, epidemiologic week of specimen collection was not found to be a confounder (data not shown).

Of the 11 AFM case-patients, 4 were infected with EV-D68, 4 were negative for enterovirus/rhinovirus according to pan-enteroviral RT-PCR, and 3 were positive according to pan-enteroviral RT-PCR; further typing of specimens from these 3 patients indicated a variety of rhinoviruses (Figure 3). One case-patient who was initially negative according to VP1 testing had a positive result on EV-D68 rRT-PCR, which was confirmed on repeat analysis. This discordance resulted from low EV-D68 RNA copy numbers in the specimen, at the limit of detection for both assays. The EV-D68 rRT-PCR cycle threshold for this specimen was 43.9 with a clear sigmoid curve. Given that we used the EV-D68 rRT-PCR for case-patients and controls, this patient was classified as EV-D68 positive.

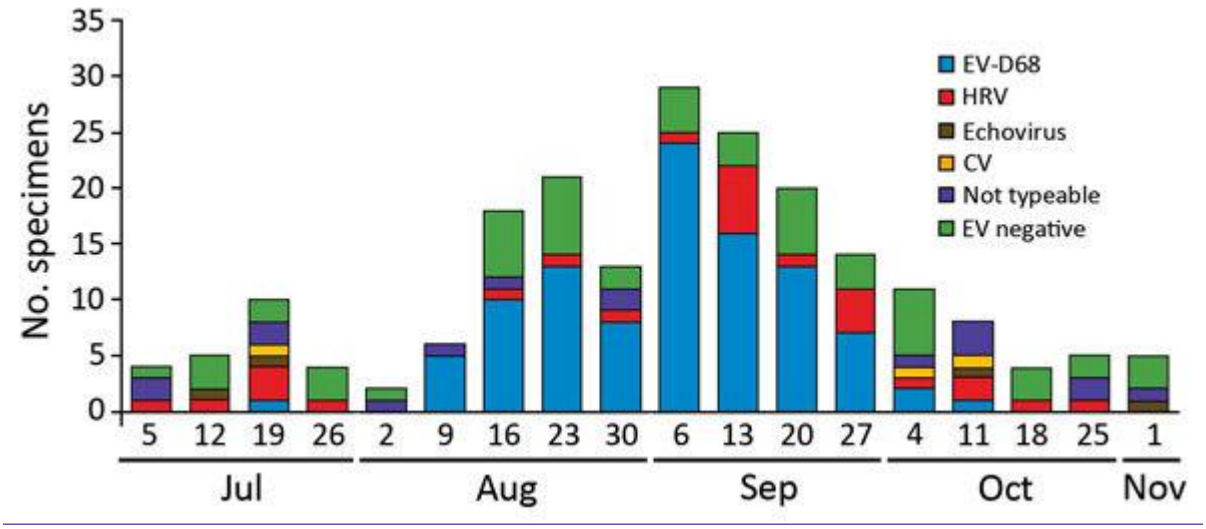

Figure 3

Discussion:

Our study demonstrated an epidemiologic association between EV-D68 infection and AFM among children during the 2014 Colorado outbreak. The odds of EV-D68 infection were 10 times higher for children with AFM than for RPP-tested controls and 4.5 times higher than for BP-tested controls. The odds of fever were also higher for AFM case-patients than for BP-tested controls; this finding was not surprising, given the clinical picture associated with pertussis. The elevated odds of EV-D68 infection for AFM case-patients compared with RPP-tested controls suggest that the prevalence of EV-D68 infection among these AFM case-patients was not likely to reflect background circulation of the virus during the outbreak. Moreover, during the outbreak, this association seems to be unique to EV-D68 because infection with other enteroviruses, rhinoviruses, or other common respiratory pathogens identified through FilmArray was not significantly associated with AFM.

Age was a confounder in this analysis. RPP-tested controls were younger, reflecting the median 5 years of age reported during the 2014 EV-D68 respiratory outbreak (18). The older age of the AFM case-patients was similar to the median 7.6 years reported in the US description of AFM cases (10). However, data from Europe and Wales describe similar disease in younger children. In a report of 3 cases of neurologic dysfunction and laboratory evidence of EV-D68 infection in Europe, these patients were 4, 5, and 6 years of age (19,20). In addition, in Wales, the ages of a cluster of 4 children with acute flaccid paralysis (3 who had respiratory symptoms preceding the acute flaccid paralysis and 2 who were positive for EV-D68) was predominantly <2 years (21). These slightly discrepant data may be indicative of the small sample size of AFM cases and highlight the need for continued surveillance to better define the epidemiology of AFM cases.

The prevalence of EV-D68 in the nasopharyngeal specimens of the controls in our study (6%–13%) was lower than expected, given the common presence of EV-D68 in PICU patients. Although the rate of EV-D68 in communities during the 2014 US outbreak is unknown, data from other EV-D68 respiratory illness outbreaks in Asia and Europe suggest a similar low prevalence rate of EV-D68 positivity in nasopharyngeal specimens: 2.3%–10.9% among hospitalized children with respiratory symptoms (2,22–25) and 2.0% among outpatients with respiratory symptoms (25). The prevalence of EV-D68 among the controls was much lower than that seen among PICU patients, suggesting either a disproportionately high acuity of EV-D68 respiratory disease testing or selective testing by clinicians in the outpatient setting compared with the intensive care setting. As the outbreak was progressing, the official CHCO respiratory illness algorithm discouraged clinicians from testing all children with respiratory symptoms seen in emergency or outpatient settings for EV-D68 because the clinical management would not change for those who were treated as outpatients. As such, children with routine respiratory symptoms seen in the emergency room, urgent care, or other outpatient clinics were not being sampled for EV-D68, and children who were infected would have been missed. We tried to account for the potential decline in outpatient testing in 2 ways. First, although we did not find week of specimen collection to be a statistical confounder, we

nonetheless included it in the multivariable model. Second, we chose an additional control group of children tested for B. pertussis. The clinical syndrome of pertussis in these children probably differed from the respiratory symptoms among children with acute respiratory illness, and the BP-tested children were probably sampled more systematically to rule out B. pertussis infection. These specimens were thus less likely to have a testing bias than were those from the RPP-tested control group. In the BP-tested controls, we saw a positive association between AFM case-patients and the presence of EV-D68.

A similar clinical presentation of some other picornaviruses lends biological plausibility to the association of EV-D68 and AFM. Enteroviruses such as enterovirus A71 (EV-A71) and poliovirus cause neurologic syndromes including acute flaccid paralysis, aseptic meningitis, and rhomboencephalitis. The MRI findings for the cluster of children in our study are similar to those induced by EV-A71 and poliovirus, both of which show tropism for the anterior horn cells of the spinal cord, although they infrequently infect the central nervous system (26,27). Similar to EV-D68, EV-A71 was initially linked to nonneurologic syndromes, specifically herpangina and hand, foot, and mouth disease, before outbreak data conclusively revealed an association between EV-A71 and neurologic syndromes. Other studies of the 2014 cluster of AFM cases have detected EV-D68 in the upper respiratory tract and, in 1 patient, in blood (10,12). However, EV-D68 is expected to be found at these sites in persons with EV-D68 respiratory illness, and detection of EV-D68 in these specimens does not prove causation of AFM. Identification of EV-D68 in cerebrospinal fluid, which provides the most definitive evidence of causation, was not reported from the 2014 cluster. Nonetheless, our study compares EV-D68 detection in AFM case-patients with detection in contemporaneous control patients with mild respiratory illness, lending additional epidemiologic support to the ecologic association between EV-D68 and AFM.

Our analysis was subject to several limitations. First, although an ideal control group would have included population-based sampling of all age-appropriate children in the Denver metropolitan area who did not have AFM during the outbreak, such a group was logistically not possible. Therefore, we used retrospective outpatient controls for whom RT-PCR diagnostic testing was performed at the discretion of providers at CHCO and specimens were retained and available for further testing. As such, our controls probably do not reflect all children in the community, and the sample might have been biased, representative only of children with mild respiratory symptoms not requiring hospitalization. The higher prevalence of EV-D68 among PICU patients suggests that prevalence in the community is higher than in the sample of children with mild respiratory symptoms for whom upper respiratory specimens were collected. However, given that most of the AFM case-patients were children with a mild respiratory prodrome, our control groups were more representative of the degree of respiratory illness seen in the case-patients than in PICU patients. Second, respiratory specimens were obtained after a much shorter interval from patients in the RPP-control group than from patients in the AFM case-patient and BP-control groups. This delay might have led to a lower prevalence of EV-D68 (or other pathogens) in these latter 2 groups than would have been found if testing had been performed sooner (28). Third, nasopharyngeal specimens are not sterile; presence of viruses in these samples might be coincidental and not causative of AFM. The association of the presence of EV-D68 in the nasopharynx and AFM might also have been biased by an unmeasured or unrecognized confounder. Fourth, RPP-negative specimens at CHCO were not sent to CDC for enterovirus/rhinovirus and EV-D68 rRT-PCR testing. Although sensitivity of the FilmArray assay is 83.7%, this test may have missed EV-D68–positive cases. Fifth, we also noted positive measures of association between non–EV-D68 enterovirus/rhinovirus exposure and AFM among both control groups, although neither association was statistically significant. However, this analysis did not have an adequate sample size to enable further exploration of this association. Last, although our models included a variable for the timing of specimen collection, because of the limited sample size of AFM case-patients we were unable to completely control for this variable through analyses that more closely matched with time of specimen collection (not shown).

In conclusion, we found an epidemiologic association between AFM and EV-D68 infection among children with respiratory illness during 2014 in Colorado. This finding goes beyond previously reported temporal associations between AFM clusters with increases in hospital admissions for respiratory symptoms and detection of EV-D68 in AFM case-patients. These epidemiologic data, combined with the biological plausibility of this association, suggest a possible causal link; however, a gap remains between the epidemiologic data and the data from extensive testing of laboratory specimens. CDC recommends continued surveillance, and a revised case definition without age restrictions has been implemented (29). For further investigation of this association, improved surveillance for AFM with timely and comprehensive specimen collection and testing for EV-D68 are needed.

Conclusion:

The most common cause in the last years is EV-D68,and is the most virus cause acute flaccid paralysis.

Future work:

Wider studies encompassing multiple regions and different ethnicities investigating EV-D68 and its correlation with acute flaccid paralysis,should be conducted to assess the role of EV-D68 and its effect,to find a vaccine.

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