Role of Enterovirus 71 in Acute Flaccid Paralysis After the Eradication of Poliovirus in Brazil

As a result of the successful initiative to eradicate poliomyelitis in the Americas, Brazil is now free of circulating wild poliovirus. The last cases of acute flaccid paralysis (AFP) with confirmed wild poliovirus isolation occurred in March 1989 (1). Since the elimination of wild poliovirus-associated poliomyelitis in Brazil and the American region by intensive mass vaccination campaigns, acute paralytic illnesses from other causes constitute a greater proportion of suspected cases. Enterovirus 71 (EV71) can cause paralytic disease with persistent flaccid paralysis (which may be confused with paralysis caused by wild polioviruses); therefore, the presence of this enterovirus in a community may complicate the evaluation of poliomyelitis control progress. Serologic evidence of EV71 infections (demonstrated by neutralization assays) has been observed in AFP patients in Brazil (9). In addition, EV71 isolates have been obtained from patients with suspected cases of poliomyelitis and their healthy contacts from different areas in Brazil and from AFP patients in Peru and Bolivia (3, Ferreira et al., in preparation).

EV71, the most recently recognized human enterovirus in the family *Picornaviridae*, has been associated with outbreaks of hand-foot-and-mouth disease and central nervous system diseases (e.g., aseptic meningitis, encephalitis, and poliomyelitis-like paralytic diseases) with persistent or transient paralysis (2,4,5). Despite some understanding of the variability in clinical manifestations and epidemiologic pattern, little is known about the contribution of EV71 to overall AFP in different countries.

Immunoglobulin M (IgM) antibody to EV71 was measured by using an μ-capture enzyme immunoassay (EIA) (6). The antigen was prepared from the BrCr strain of EV71, and biotinylated anti-EV71 monoclonal antibody as detector was also prepared (6). The results were expressed as the difference in mean optical density values measured in triplicate wells of positive antigen (P) and negative controls (N). A specimen was considered positive if the observed P-N was ≥ 2 standard deviations (SD) above the mean of the optical densities for the negative control serum (P-N ≥ 0.25).

Serum samples were obtained from 92 infants from different regions of Brazil who had suspected poliomyelitis and symptoms of AFP during 1989 and 1990. The 138 samples included paired serum samples (S1 and S2) from 46 patients (92 sera) and 46 serum samples (S1 only) from 46 patients. Acute-phase serum samples (S1) were obtained 1 to 15 days after the onset of symptoms, and a second group of samples were obtained (S2) 15 to 45 days later; all were from children whose stool specimens were negative for wild poliovirus.

To check the assay reagent cross-reactivity with polioviruses, all 138 serum specimens were tested in parallel by the same described method, but by using poliovirus serotypes 1, 2, and 3 Sabin strains instead of EV71 as the antigen. The assay had also been checked in a limited fashion for heterotypic response due to poliovirus infection by testing sera from a limited number of known vaccine-associated paralytic poliomyelitis cases (4). These vaccine associated case sera showed no evidence of IgM response to EV71 antigen from poliovirus infection.

Specimens from 20 of 92 patients with suspected cases of poliomyelitis had positive IgM responses to EV71 (Table). Three patients (2287, 3805, and 3489) whose specimens were positive had only a single serum specimen (S1) available. Four serum specimens (1819, 2018, 1918, and 1906) only had positive IgM titers for the first serum (S1), and five specimens from Bahia State (429, 784, 1130, 1325, and 780) had an IgM response only for the convalescent-phase sera (S2). The IgM-positive AFP specimens were from residents of eight widely dispersed regions of Brazil with eight specimens collected in 1989 and 12 collected in 1990. Six of 19 AFP patients with IgM positive results had residual paralysis after 60 days, which is considered to be clinically characteristic of poliomyelitis due to poliovirus. The remainder of the AFP patients only had a transient paralysis from which they recovered.

The presence of residual paralysis and EV71 IgM antibodies in 20 (21.7%) of the 92 AFP patients from eight geographic regions indicates that EV71 may be causing AFP-like poliomyelitis throughout Brazil. The incidence of EV71 infection associated with AFP in Brazil is high when compared with its incidence in the United States, where EV71 infections leading to AFP are very uncommon but have rates not much lower than those of vaccine-associated paralytic poliomyelitis (5,8). Poliomyelitis-like paralytic illnesses in Brazil in 1989 and 1990 are not likely attributable to polioviruses; most of these cases occurred after the last confirmed case of wild poliovirus paralysis, and poliovirus was not isolated from any patient's stool specimens. EV71 causes persistent flaccid paralysis; our results link EV71 with

Table. Data from patients positive for enterovirus 71(EV71)

Case	Initial symptom	Brazilian state	EV71 IgM S1	EV71 IgM S2	Year	Sequelae after 60 days
429	AFP	BA	-	+	1989	Yes
784	AFP	BA	-	+	1989	No
1130	AFP	BA	-	+	1989	No
1325	AFP	BA	-	+	1989	Yes
780	AFP	BA	-	+	1989	NF
2287	AFP	BA	+	NA [a]	1990	No
907	AFP	DF	+	+	1989	No
1014	AFP	DF	+	+	1989	No
1394	AFP	RS	+	+	1989	Yes
2593	AFP	RS	+	+	1990	Yes
1826	AFP	SC	+	+	1990	No
1819	AFP	SC	+	-	1990	Yes
1815	AFP	SC	+	+	1990	Yes
1809	AFP	SC	+	+	1990	No
1940	AFP	PE	+	+	1990	No
2018	AFP	RN	+	-	1990	No
1918	AFP	PB	+	-	1990	No
1906	AFP	RN	+	-	1990	No
3805	AFP	RJ	+	NA	1990	No
3849	AFP	RJ	+	NA	1990	No

BA = Bahia State; DF = Distrito Federal; RS = Rio Grande do Sul; SC = Santa Catarina; PE = Pernambuco; RJ = Rio de Janeiro; RN = Rio Grande do Sul; PB = Paraiba.
[a]NA = not available; NF = no follow-up.

some of these AFP cases in Brazil. Subsequent testing on specimens from some of these patients resulted in the isolation of EV71; neutralizing antibodies were found with seroconversion (3, 9,Ferreira et al., in preparation). Before the eradication of wild polioviruses in Brazil, all AFP patients who had residual paralysis as described in this study would have generated concern about undetected wild poliovirus circulation because their cases resembled poliovirus-caused paralysis.

The pattern of results obtained with the acute-phase and convalescent-phase sera in this study is not uncommon for serologic tests of enterovirus infections. Only seven patients with AFP had two positive serum specimens; for nine AFP patients, only one of the two specimens was positive. Some S1 samples were collected during the early stages of the disease when the IgM titers may not have been detectable. Our results are consistent with seroconversion data as has been observed with enterovirus 70 infections and a similar assay (7); however, neutralizing antibodies were detected in this study's convalescent-phase sera (9). Additionaly, late specimen collection or inadequate specimen storage or handling before receipt in the laboratory might have affected IgM titers adversely because these conditions were not carefully controlled. Although cross-reactivity of antibody from other nonpolio enteroviruses cannot be excluded, the possibility can only be addressed directly by additional testing with other serologic assays. However, when the same assay was used to examine specimens from known cases of vaccine-associated paralytic poliomyelitis from the United States, no heterotypic response was observed (4).

Since 1990, for technical and operational reasons, serologic methods have not been used to confirm cases of AFP as caused by wild poliovirus; therefore, serum samples are no longer available for routine poliovirus diagnosis. Our recent efforts have been directed towards isolating and characterizing nonpolio enteroviruses (with emphasis on EV71) from patients with suspected poliomyelitis and towards examining the geographic and temporal relationship between these AFP cases.

Public health personnel and pediatricians should be alerted to the possible role of nonpolio enterovirus infections in the differential diagnosis of AFP or other severe central nervous system diseases, particularly in areas where the circulation of wild poliovirus has been interrupted. The laboratory diagnosis of all AFP cases should routinely include tests capable of detecting EV71 as well as other enteroviruses once the primary objective of poliovirus eradication has been achieved.

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References

1. de Quadros CA, Andrus JK, Olivé JM, da Silveira CM, Eikhof RM, Carrasco P, et al. Eradication of poliomyelitis: progress in the Americas. Pediatr Infect Dis J 1991;10:222-9.
2. Melnick JL. Enterovirus type 71 infections: a varied clinical pattern sometimes mimicking paralytic poliomyelitis. Rev Infect Dis 1984; 6(S2):S387-S90.
3. da Silva EE, Fillipis AM, Cortes LC, Schatzmayr HG. Isolation of enterovirus 71 from suspected poliomyelitis cases and their healthy contacts in Brazil. Abstracts of the VIIIth International Congress of Virology 1990; P22-067.
4. Nagy G, Takátsy S, Kurán E, Mihály I, Dömök I. Virological diagnosis of enterovirus type 71 infections: experiences gained during an epidemic of acute CNS diseases in Hungary in 1978. Arch Virol 1982;71:217-27.
5. Hayward JC, Gillespie SM, Kaplan KM, Packer R, Pallansch MA, Plotkin S, et al. Outbreak of poliomyelitis-like paralysis associated with enterovirus 71. Pediatr Infect Dis J 1989;8:611-6.
6. Helfand RF, Khan AS, Pallansch MA, Alexander JP,

Meyers HB, DeSantis RA, et al. Echovirus 30 infection and aseptic meningitis in parents of children attending a child care center. J Infect Dis 1994;169:1133-7.
7. Bern C, Pallansch MA, Gary HE, Alexander JP, Török TJ, Glass RI, et al. Acute hemorrhagic conjunctivitis due to enterovirus 70 in American Samoa: serum-neutralizing antibodies and sex-specific protection. Am J Epidemiol 1992;136:1502-6.
8. Alexander JP, Baden L, Pallansch MA, Anderson LJ. Enterovirus 71 (EV71) infections and neurologic disease–United States, 1985-1989. J Infect Dis 1994; 169:905-8.
9. da Silva EE, Fillipis AM, Schatzmayr HG, Cortes LC. Evidence of enterovirus 71 infections in Brazil. Mem Inst Oswaldo Cruz 1990;85:131-2.