J Microbiol Immunol Infect
2001;34:167-170

Duration of enterovirus shedding in stool

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Received: October 6, 2000 Revised: October 27, 2000 Accepted: December 22, 2000

Excretion of enterovirus (EV) may persist for months after an EV infection; the exact duration of excretion, however, is not yet known. Twelve children who were infected with EV between September 1998 and June 1999 were enrolled into this study. The patients included 4 boys and 8 girls, aged from 1 month to 5 years. Six patients were asked to join this virus isolation program, and the other 6 were followed-up regularly. Only 2 of the patients were infected with EV 71. To delineate the duration of EV shedding in each case, throat swabs for virus isolation were performed every 1 or 2 weeks for at least 1 month, and stools were analyzed for at least 2 months following the same schedule. After the infection, EV was identifiable in the throat in 4 patients for 1 to 2 weeks. Excretion of EV through stool was evidenced for up to 7 weeks in 6 patients, 8 weeks in 3, and 11 weeks in 1. In the 2 patients who failed to show up for follow-up visits from the 7th week, excretion of EV through stool was recorded for at least 7 weeks. Different serotypes of EV could be isolated from the same patient who was not experiencing febrile illness in 2 instances in a series of virus cultures. Coexistence of vaccine poliovirus and non-polio EV, both isolated from stool, was evidenced in 2 patients. Results from this study suggest that EV may not be identified from the throat 2 weeks after the infection, but its excretion through stool can persist for up to 11 weeks. This study also demonstrated that subclinical EV coinfection could occur, and that live vaccine poliovirus did not interfere with the invasion of other non-polio EV.

Key words: Enterovirus, poliovirus, vaccine, viral shedding

Enteroviruses (EV) are made up of at least 67 serotypes, and are among the most common and significant causes of infectious disease in infants and children. They are associated with a broad spectrum of clinical diseases, which include aseptic meningitis, herpangina, hand-foot-mouth disease (HFMD), conjunctivitis, pleurodynia, myopericarditis, poliomyelitis, various exanthems, and nonspecific febrile illness.

Hand-foot-mouth disease, which is usually caused by coxsackievirus A16 (cox A16), is a common illness in children. Patients who had contracted HFMD usually recover without complications within 5 to 7 days. During an EV epidemic in 1998 in Taiwan, however, a number of complications of HFMD such as aseptic meningitis, encephalitis, poliomyelitis-like syndrome, and even fatal pulmonary edema were documented. These episodes were later proved to be caused mainly by EV 71 [1-4].

Outbreaks of EV 71 infection caused more than 20 deaths in Bulgaria (1975), Hungary (1978), and Malaysia (1997) [5-8]. In Taiwan, however, an epidemic of HFMD or herpangina caused by EV 71 with high morbidity and mortality rates had never occurred before 1998. The outbreak in 1998 resulted in panic in Taiwan, but the pathogenesis of the pulmonary edema caused by EV 71 was not clearly identified, and a method of preventing further transmission of EV 71 was not implemented. The fecal-oral route is probably the most common mode for transmitting EV, augmenting hand washing after contact with contaminated material may therefore help limit transmission .

In patients who have received oral poliovirus vaccine, the virus shedding persists in the throat for 1 to 3 weeks, and is excreted through the stool for 1 to 6 weeks or longer [9]. Most coxsackieviruses and echoviruses can be isolated from stool even at 2 to 3 months after a nonspecific illness [9-13]. No information is available concerning the duration of the shedding of non-polio EV from stools after infection. This study aimed to delineate the exact duration of the shedding of EV other than poliovirus in the throat and in stool, and to further define the period of possible fecal-oral transmission.

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Materials and Methods

From September 1998 through June 1999, 25 children with proven EV infections at the Pediatric Department of the Chang Gung Children's Hospital were enrolled in the study. The patients aged from 1 month to 5 years; none of them have underlying diseases. They were categorized into the following 2 groups: group 1 comprised of children who presented with HFMD, herpangina, prolonged fever, and fever with seizure, as well as young infants with fever according to the microbiology laboratory logs. Group 2 included children who presented with HFMD and herpangina, as well as young infants with fever or aseptic meningitis. Six patients were included in group 1; EV was identified from throat swabs in all 6 patients, and from rectal swabs in 3. The patients were asked to attend follow-up sessions; starting from the 5th week after infection, stool samples were obtained and sent for virus isolation every 1 or 2 weeks for at least 1 month. A total of 19 patients were included in group 2, and all were followed-up prospectively. Throat swabs for virus isolation were obtained every 1 or 2 weeks for at least 1 month; stool samples were obtained at the same weekly or biweekly intervals for at least 2 months.

Samples were inoculated into MRC-5 (human embryonic fibroblast), LLC-MK2 (monkey kidney cell), Hep-2 (laryngeal carcinoma cell), and RD (rhabdomyosarcoma) cell cultures. Once an enteroviral cytopathic effect that involve more than 50% of the cell monolayer were detected, the cells were scraped, and indirect fluorescent antibody staining with panenteroviral antibody (Chemicon International, Temecula, CA, US) was performed to identify the EV. The isolates were also screened for EV 71 by immunofluorescence with EV 71 monoclonal antibody (Chemicon International). The isolates were identified as poliovirus type 1 to 3 (polio 1-3); coxsackievirus A9 and A16 (cox A9 and A16); coxsackievirus B1 to B6 (cox B1-6); and echovirus 4, 6, 9, 11, and 30 (echo 4, 6, 9, 11, and 30) by an immunofluorescent assay and/or standard neutralization techniques. Enteroviruses that could not be further identified at the serotype level via the available monoclonal antibodies at the microbiology laboratory were regarded as panenterovirus.

Results

Three boys and 3 girls (patients 1-6) aged from 1 month to 5 years were included in group 1. At least 3 (range, 3-6) specimens of stool were obtained from each patient during the follow-up period. A total of 29 specimens of stool were collected from group 1. Results of cultures that were positive for EV were noted in 14 specimens from 4 patients, and 4 serotypes were identified. The detailed results are shown in Table 1. In patients 1 and 2, no positive results were seen from the 7th week after infection. In patient 3, EV shedding in stool was not identified in the 6th week but in the 7th and the 8th week (panenterovirus) after infection; the results were

Table 1. Results of virus isolation from stools in groups 1 and 2

Patient no.	Week												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1	Cox A16						N	N	N				
2	Cox A16						N		N	N			
3	panEV					N	panEV	panEV	N		N		
4	panEV						N	panEV	N		N	panEV	panEV
5	panEV					panEV	panEV	panEV	N	N		N	
6	panEV						Polio + Echo 4	Polio + Echo 4	Polio	Polio	Polio	Polio	
7	EV 71		EV 71	N	N	N	N						
8	Cox A16		Cox A16			Cox A16							
9			Cox A16	Cox A16	N	Cox A16	N	N	N				
10	Cox B2 or Echo 4[a]		Cox B2 or Echo 4	N	panEV	N	N	N	N				
11	EV 71			EV 71		EV 71					N		
12	Cox A16	Cox A16		N	N	Polio	Polio	Polio + Cox A16	Polio		Cox A16	N	

Abbreviations: Cox A16 = coxsackievirus A16; Cox B2 = coxsackievirus B2; Echo 4 = echovirus 4; EV 71 = enterovirus 71; N = no growth; panEV = panenterovirus; Polio = poliovirus

Note: group 1 = hand-foot-mouth disease, herpangina, fever, and seizure
group 2 = hand-foot-mouth disease, herpangina, and aseptic meningitis

[a]Virus isolation of cerebrospinal fluid.

negative thereafter. In patient 4, who was initially infected with panenterovirus, EV was not isolated in the 7th week after infection, but panenterovirus was identified from stool samples in the 8th week. After an acute tonsillitis in the 11th week, panenterovirus was identified from stool in the 12th and 13th week in this patient. The EV shedding (panenterovirus) in stool persisted until the 8th week after infection in patient 5. Patient 6 received a first dose of oral poliovirus vaccine in the 6th week after infection. Two serotypes of EVs, poliovirus and echo 4, were isolated from the stool specimens of this patient between the 7th and the 12th week after infection. During the follow-up period, febrile illness was noted only in patient 4, who has experienced acute tonsillitis in the 11th week.

Of the 19 patients (aged from 1 month to 5 years) who were enrolled into group 2, only 6 (1 boy and 5 girls, patients 7-12) completed the study. The 13 patients who were followed up for less than 1 month were excluded. At least 3 (range, 3-9) stool specimens and 1 (range, 1-6) specimen of throat swab were obtained for virus isolation during the follow-up period from each patient. A total of 37 stool specimens, 24 specimens of throat swab, and 1 specimen of cerebrospinal fluid were obtained from these 6 patients. Positive results for EV were evidenced in 21 stool specimens, 7 specimens of throat swab, and 1 specimen of cerebrospinal fluid, and 6 serotypes of EV were identified. Detailed results are shown in Tables 1 and 2. During the follow-up period, febrile illness was noted only in patient 12, who had an acute bronchiolitis in the 6th week after infection and was treated at the outpatient department of the hospital. Patients 7 and 11 were the only 2 cases infected with EV 71 in this study. In patient 7, the positive results for EV 71 from stool specimens persisted for 3 weeks after infection; another serotype of EV, panenterovirus, was isolated from the throat swab in the 6th week. In patient 8, positive results from the stool specimens for cox A16 were noted in the 6th week after infection, but she then failed to continue follow-up visits thereafter. In patient 9, a positive result from throat swab for cox A16 was evidenced in the 1st week after infection, and positive results from stool specimens for cox A16 persisted until the 6th week. In patient 10, the positive result from cerebrospinal fluid (cox B2 or echo 4 due to the cross-reactivity in the immunofluorescent assay) was evidenced in the 1st week after infection, but no EV was isolated from throat swabs. Positive results for cox B2 or echo 4 and panenterovirus were evidenced in stool specimens at the 3rd and the 5th week, respectively. In patient 11, a positive result for EV 71 was identified in a throat swab at the 1st week after infection, and positive results from stool specimens for EV 71 persisted for 6 weeks; she then failed to continue follow-up visits for 1 month. In patient 12, who was infected with cox A16 and received the first dose of oral poliovirus vaccine in the 5th week, 2 serotypes of EV, cox A16, and poliovirus were isolated from stool in the 8th week after infection. Positive results for cox A 16 persisted until the 11th week after infection in this patient.

Discussion

This study was conducted after the major outbreak of EV 71, and only 2 patients included in this study were infected with EV 71. Results from this study indicated that EV may not be identifiable from throat 2 weeks after illness, whereas the duration of its excretion from stools may range from 3 to 11 weeks, although a 4- to 8-week duration is more common. Because the fecal-oral route is probably the most common mode for EV transmission, augmenting hand washing is important in interrupting viral transmission, and should be emphasized throughout the entire period of fecal EV excretion, which may persist for up to 11 weeks after illness. Transmission via saliva or the respiratory droplets, however, cannot be disregarded during the acute phase of the illness when EV can be identified in the throat.

During the EV 71 epidemic in Taiwan in 1998, some clinicians suggested that infection with one kind of virus

Table 2. Results of virus isolation from throat swabs in group 2

Patient	Week							
no.	1	2	3	4	5	6	7	8
7	N	N	N	N	N	panEV		
8	Cox A16							
9	Cox A16		N	N	N			
10	N		N	N	N			
11	EV 71	N				N		
12	Cox A16			N	Polio	Polio	N	N

Abbreviations: Cox A16 = coxsackievirus; EV 71 = enterovirus 71; N = no growth; panEV = panenterovirus; Polio = poliovirus
Note: group 2 = hand-foot-mouth disease, herpangina, and aseptic meningitis

may interfere with the invasion of another kind of virus in the same patient. Thus, it was assumed that the universal administration of oral poliovirus vaccine to the susceptible population, a method that had been used in Bulgaria in 1975, may help terminate the epidemic [6]. Results in this study, however, highlighted the fact that different serotypes of EV can be isolated serially from the same patient who were not experiencing febrile illness, and indicated the possibilities of subclinical EV reinfection. In addition, the coexistence of vaccine poliovirus and non-polio EV in 2 patients also suggested that poliovirus vaccine does not interfere with the invasion of other non-polio EV. Neither of these results supports the use of poliovirus vaccine to control an EV 71 epidemic.

The number of cases, serotypes of EV identified, and patients infected with EV 71 were too small in this study to allow an accurate measurement of the exact duration of the shedding of EV, particularly EV 71, in the throat and the stool. Further study is needed to elucidate this issue.

In summary, EV was identified from the throat for the first 2 weeks after infection, whereas EV excretion from stools can last for up to 11 weeks. Subclinical EV reinfection is not uncommon in patients with EV infection. Live poliovirus vaccine does not seem to interfere with the invasion of the other non-polio EV.

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