

ENTEROVIRUSES AT A TERTIARY CARE CENTER IN SAUDI ARABIA, 1989-1995

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Enteroviruses (EVs) are among the most common viral pathogens affecting humans. Enterovirus (EV) infections occur worldwide in temperate climates with a marked summer/fall season and have a high year-round incidence in tropical and subtropical areas. In Saudi Arabia, the epidemiology of enteroviruses is unknown. Of the clinical specimens submitted for diagnosis during a six-year period (1989-1995) at King Faisal Specialist Hospital and Research Centre (KFSH&RC) in Riyadh, a wide variety of diseases were associated with the isolation of enteroviruses. Among the diseases were herpangina, sepsis-like illness, hand-foot-and-mouth syndrome, laryngotracheitis (croup), aseptic meningitis, pneumonia, and gastrointestinal illness. During the six-year study, a variety of enteroviruses were isolated. Although epidemics were seen in mid-winter and early spring, EV which may cause sporadic diseases were isolated in each of the 12 months. Health care providers must become aware of the epidemic potential not only in mid-winter and early spring, but also throughout the year, as this will aid in the diagnosis and management of this illness. *Ann Saudi Med 1997;17(1):16-19.*

Enterovirus (EV) infections are responsible for significant and frequent human illness throughout the world. Control of polioviruses in much of the world, including Saudi Arabia, has focused attention on the nonpolio EV. The nonpolio EVs (echoviruses, coxsackieviruses A and B and the newer EVs) are exceedingly common, and the spectrum of disease is protean. Recent data for the United States has shown that nonpolioenteroviruses are estimated to cause 10 to 15 million systemic infections annually.^{1,2} Little is known about the epidemiology of these infections in Saudi Arabia, the Gulf Region, and the Middle East.^{3,6} In an attempt to learn more about the nature of EV infection in Saudi Arabia, we reviewed the data on enterovirus infection over a six-year period (May 1989 through May 1995). Clinical specimens submitted from symptomatic patients for viral diagnosis at KFSH&RC in Riyadh have underlined the ubiquitous nature of enteroviruses in our community. A variety of clinical diseases caused by different serotypes was seen during this period. This report documents these findings and it not only determines the frequency and seasonal variation of EV infections in the Saudi population, but also emphasizes the importance of disease caused by EVs throughout the year.

Material and Methods

Study population specimens for viral diagnosis were obtained from patients seen at KFSH&RC in Riyadh. Patients ranged in age from younger than one month to age 70 years; all were symptomatic. The majority of the specimens examined were obtained from infants and children.

Collection and Processing of Specimens for Viral Culture

Clinical specimens were received in the form of nasopharyngeal aspirate in a sterile container (throat swab on a sterile cotton tip, immediately placed in 1.2 mL viral transport medium Virocult, Medical Wire and Equipment Company, New York), fecal specimen or undiluted cerebrospinal fluid (sterile tube). Throat, nasopharyngeal aspirate and fecal specimens were centrifuged at 2700 rpm for 10 minutes. CSF was inoculated without centrifugation. Serum and mononuclear preparations were processed from whole blood using neutrophil isolation media (Cardinal Associates, Sante Fe, NM).

Virus Isolation and Identification

Clarified nasopharyngeal aspirate, throat and fecal specimens, CSF, serum and mononuclear cell suspension (0.2 mL) were inoculated into one tube of four cell lines:^{7,8} human lung adenocarcinoma cells (A549), African Green Monkey kidney (Vero), human foreskin fibroblast cells (HFF) and secondary rhesus monkey kidney (LLC-MK2). Cultures were observed for up to 28 days for the appearance of cytopathic effect (CPE). Cultures were read

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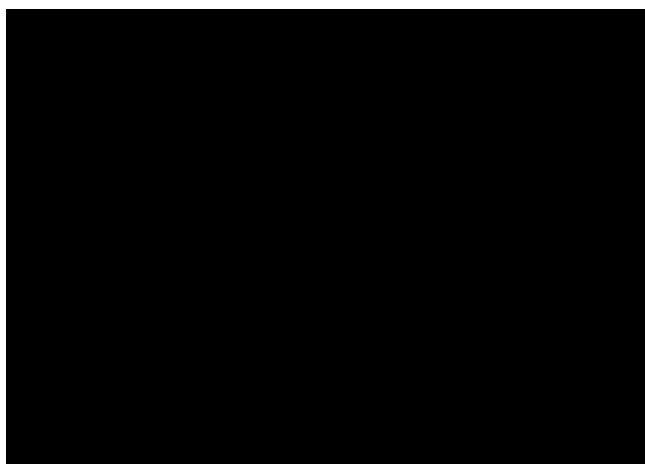


FIGURE 1. Total number of isolates of enterovirus by month at King Faisal Specialist Hospital and Research Centre, 1989-1995.

the day after inoculation to check for both CPE and contamination or toxicity problems. If neither of the above was noted, they were read routinely three times per week. Isolates producing typical CPE were identified by neutralization with the Lim Benyesh-Melnick equine antiserum pools and confirmed by neutralization with nonspecific antisera.^{7,9} Isolates that were not neutralized were shown to be EVs by using standard methods for testing for acid and ether stability.⁸

Results

From May 1989 through May 1995, a total of 6390 specimens were examined and 88 of the specimens yielded enteroviruses (Table 1). The same agent isolated from consecutive specimens from a patient was counted as only one infection. The seasonal distribution of enteroviruses for the study period is shown in Figure 1. The numbers of isolates peaked during the months of December through April, although at least one isolate was obtained during each month of the year (Figure 1). The frequency with which the various serotypes were isolated is shown in Table 2. Echovirus 6 was the most frequently isolated agent, followed by coxsackievirus B₃ and B₄. The majority of enterovirus infection occurred in preschool children. The rate of infection was highest in the one- to four-year-old group and showed significant decline in teenage years and adulthood (Table 2). The female-to-male ratio of enteroviral infection in an infant was 1.1:1. The predominance of females is limited to this group and the overall ratio for the combined age groups to age 70 favored male predominance 1.2:1 (Table 3). It is evident from the data that clinical syndromes such as pneumonia and aseptic meningitis can be caused by more than one serotype of enterovirus and also that a particular

TABLE 1. The isolation year and associated clinical syndromes of enteroviruses at KFSH&RC in Riyadh.

Year	No. of isolates	Enterovirus serotypes	Associated clinical syndrome
May-Dec 1989	2	Untyped (2)	Upper respiratory infection, fever
1990	4	Untyped (2), poliovirus 2(2)	Upper respiratory infection, fever, diarrhea
1991	6	Echovirus 6(1); coxsackievirus B1(1), B3(2); untyped (2)	Aseptic meningitis (ECHO 6), herpangina (B1), sepsis-like illness (B3), pharyngitis (B3), diarrhea
1992	12	Echovirus 15(2), 25(2); coxsackievirus A16(1); coxsackievirus B1(1); poliovirus 2(2), 3(1); untyped (3)	Upper respiratory infection (ECHO 15), pharyngitis (ECHO 25), hand-foot-and-mouth (A16), pharyngitis (B1), diarrhea, aseptic meningitis (untyped), fever
1993	11	Echovirus 9(1), 11(2); coxsackievirus A16(1); coxsackievirus B1(1), B4(2); untyped (4)	Pneumonia (ECHO 9), gastrointestinal illness (ECHO 11), aseptic meningitis (A16), pharyngitis (B4)
1994	24	Echovirus 2(1), 4(1), 6(2), 17(2), 19(1), 30(4); coxsackievirus B2(1), B3(4), B4(2), B5(1); poliovirus 1(1); untyped (4)	Aseptic meningitis (ECHO 2, 30), pharyngitis (ECHO 4), gastrointestinal illness (ECHO 17, 19), fever, rash (ECHO 2, 6), pneumonia (B4), diarrhea (B5), fever
Jan-May 1995	29	Echovirus 6(8), 11(1), 24(3); coxsackievirus B4(2); poliovirus 2(2), 3(1); untyped (12)	Aseptic meningitis (ECHO 6), pneumonia (ECHO 6, 11, cox B4), upper respiratory infection (ECHO 24), croup (cox B4), diarrhea, fever, rash
Total	88		

enterovirus, such as coxsackievirus B₄, can cause different clinical syndromes of pneumonia, croup and pharyngitis (Table 1). Sepsis-like illness was seen in a neonate who presented at day eight of life with fever, lethargy, cyanosis associated with CSF pleocytosis and disseminated intravascular coagulation (DIC). All bacterial cultures (blood, CSF, urine) were negative, but the nasopharyngeal aspirate was positive for coxsackievirus B₃. Viremia was detected in three patients with leukemia during febrile illness, blood culture was positive for echovirus 6 and urine was also positive in one patient. Although poliovirus types 1, 2 and 3 were isolated from children with mild febrile illness or diarrhea, the patients' histories clearly indicated that these isolates were vaccine associated.

TABLE 2. Frequency of isolation of individual serotypes of enterovirus.

	Serotype	No. of isolations	Total
ECHO	2	1	31
	4	1	
	6	11	
	9	1	
	11	3	
	15	2	
	17	2	
	19	1	
	24	3	
	25	2	
	30	4	
Coxsackie	A16	2	2
Coxsackie	B1	3	17
	B2	1	
	B3	6	
	B4	6	
	B5	1	
Polio	1	1	9
	2	6	
	3	2	

TABLE 3. Frequency of enterovirus isolation by age and sex.

Age of patients	Positive		Total
	Male	Female	
0-4 months	4	6	10
4-8 months	6	8	14
8-12 months	6	4	10
1-4 years	20	17	37
4-8 years	4	3	7
8-12 years	2	1	3
12-16 years	1	1	2
20-40 years	4		4
40-60 years			
60-70 years	1		1
Total	48	40	88

Furthermore, all of these children had spontaneous resolution of their febrile illness without paralysis or other complications.

Discussion

The epidemiology of EV infection has not been well studied in the Middle East and Arab Gulf region. Early and recent reports have concentrated on the identification of wild polioviruses and the failure of attenuated trivalent oral poliovaccine (TOPV) to give adequate protection

against infection.³⁻⁶ Information about the epidemiology and spectrum of diseases associated with nonpolio EV is not available in our region. This may be due to the difficulty in maintaining cell culture in some laboratories, the complexity and expense of their definite identification and the unavailability of rapid methods for their detection in clinical specimens outside the research laboratory setting.¹³ During the six-year study, the seasonal distribution of EVs in Saudi Arabia was the exact opposite of that observed in most temperate countries in the northern hemisphere.^{1,10,12,13} EVs were a cause of an array of clinical diseases in our community, with a high infection rate in mid-winter and early spring. Also less well known in this country is the fact that enteroviruses may cause sporadic disease throughout the year; at least one enterovirus was isolated from clinically symptomatic patients during every month of the year. Thus, the time of the year should not exclude the consideration of enteroviruses as a possible etiologic agent of syndromes which may, in fact, arise from enterovirus infection.

Infants and young children make up a substantial portion of the enterovirus-susceptible population.^{2,10,11} As many as 84% of infants and preschool children in this study were found to be infected with enteroviruses. At least 18 enterovirus serotypes were isolated from clinical specimens cultured during the study period. Echoviruses represent slightly more than half of these clinical isolates and Group B coxsackieviruses represent about one-fourth. It is likely that Group A coxsackievirus are considerably underrepresented in this study, because only a few serotypes, such as A₉ and A₁₆, grow readily in cell culture. This finding is similar to the nonpolio EV serotypes encountered in surveys done in the United States.^{1,12,13} The increased frequency of isolation of enterovirus in our laboratory from two isolates in 1989 to 29 isolates in the first six months of 1995 may be due, in part, to the increase in awareness of our medical staff about nonpolio EV infection. (We have seen a significant increase in the number of clinical specimens submitted for viral culture at the beginning of this year.) Another possibility is the increased numbers of nontypable enteroviruses during this period, which may represent an epidemic with a new type of enterovirus in our community.

EVs are not usually associated with significant morbidity or mortality in immunocompetent individuals. On the other hand, neonates and immunocompromised individuals are well-recognized groups that are particularly prone to the development of severe EV disease.¹⁴⁻¹⁶ In our study, a wide spectrum of symptomatic enteroviral disease was seen. This included mild disease such as upper respiratory infection, pharyngitis, rash and fever, to more severe disease, such as aseptic meningitis, sepsis-like illness, disseminated infection and pneumonia, which all required hospital admission. The neonate with sepsis-like

illness presents a good example of the severity of this illness in this age group, which has been well known and documented in previous studies^{14,15} (this patient survived after a stormy hospital course). Viremia was a marker of disseminated disease in three patients with leukemia; two patients survived, but the third died with dual viral and gram-negative sepsis.

In the future, it may be important to carefully monitor the behavior of all of the enteroviral agents, in an attempt to anticipate enterovirus activity, perhaps to avert infection, and to diagnose more rapidly those illnesses that occur.

The recent advances toward the adaptation of nucleic acid-based detection systems to clinical EV diseases^{17,18} and the promising anti-EV compounds developing^{19,20} will aid the clinician in rapid diagnosis and the use of specific antiviral therapy. Efforts should be made in the future to do larger prospective studies in this region of the world for a better understanding of the epidemiology of EV infection among both symptomatic and asymptomatic patients.

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