J Microbiol Immunol Infect
2000;33:1-8

Enterovirus infections with special reference to enterovirus 71

G.D. Hsiung[1,2], Jen-Ren Wang[3,4]

[1]Department of Laboratory Medicine, Yale University School of Medicine, New Haven CT, and [2]VA Connecticut Healthcare System, West Haven, CT, USA; [3]Department of Medical Technology, College of Medicine, National Cheng Kung University and [4]NHRI Tainan Virology Laboratory for Diagnosis and Research, National Cheng Kung University Hospital, Tainan, Taiwan, ROC

Received: November 8, 1999 Accepted: November 20, 1999

The enteroviruses comprise a large group of immunologically distinct serotypes of viruses belonging to the family of Picornaviridae. Many enteroviruses cause diseases in human, but the infections are generally mild as asymptomatic, therefore, enteroviruses are considered to be unimportant as human pathogens. However, enteroviruses may also result in serious or even fatal disease (as shown in the enterovirus 71 (EV71) epidemic in Taiwan in 1998). There are three types of polioviruses, Coxsackievirus group A and group B viruses, and echoviruses group. All together a total of 67 types are available. Starting from enterovirus type 68 to 71, they are named as enterovirus types. Enterovirus type 72 is hepatitis A virus. Paralytic disease of poliomyelitis was recorded in ancient time but characterization of poliovirus was not reported until the turn of the 19th century that poliomyelitis was a viral disease. The major breakthrough for diagnosing and controlling of poliomyelitis was the discovery that poliovirus can be propagated in human embryonic tissues in cultures. As soon as cultures of human and monkey cells began to use for isolating polioviruses in stool specimen of patients, more unknown viruses were isolated which unlike polioviruses nor Coxsackie viruses; they were called "orphan" viruses or human enteric viruses, name later simplified to "echoviruses". Morphologically all enteroviruses are alike. They are small, ether insensitive viruses with an RNA genome. Their nucleic acid is single stranded, and the nucleocapsid has a cubic (icosahedral) symmetry, and is naked. The host ranges of enteroviruses vary greatly from one type to the next and even among strains of the same type. Polioviruses have a very restricted host range among laboratory animals. Virus isolation is the best method for diagnosis of enterovirus infection, but infection in the central nervous system (CNS) may be detected by polymerase chain reaction (PCR). Currently final identification and serotyping of enteroviruses are by indirect immunofluorescent tests using monoclonal antibody or by neutralization test using antiserum pools described by Lim and Benyesh-Melnick. The incidence and prevalence of diseases associated with the enterovirus infections are varied. The circulation of enteroviruses recently in Tainan and the epidemic of EV71 in Taiwan in 1998 are described in this review. Although poliovirus infection may be eradicated from the world due to the efficient vaccination program, there is no specific antiviral agents for either treatment or prevention for other enterovirus infections. In 1991, a new antiviral "pleconaril" which is a novel orally bioavailable and systematically acting small molecule inhibitor for picornaviruses. "Pleconaril" is currently in clinical trials for treatment of enterovirus meningitis and respiratory infections.

Key words: Enterovirus, enterovirus 71 (EV71), Picornaviridae

The enteroviruses comprise a large group of immunologically distinct serotypes of viruses belonging to the family of Picornaviridae. In 1963, the name picornaviruses ("pico" = small, "rna" = ribonucleic acid genome) was introduced as a larger grouping which includes not only the enteroviruses but also the rhinoviruses because these viruses had fundamentally similar properties. The International Committee on Taxonomy of Viruses officially assigned family status (Picornaviridae) to this larger group [1].

Many enteroviruses cause diseases in human, but the infections are generally mild as asymptomatic and as a result, enteroviruses are considered to be unimportant as human pathogens. However, enteroviruses may also result in serious or even fatal disease (see later section on enteroviruses 71 epidemic in Taiwan in 1998).

Corresponding author: Dr. Jen-Ren Wang, Department of Medical Technology, National Cheng Kung University, One University Road, Tainan, 701, Taiwan, ROC.

Classification of Enteroviruses

All enteroviruses belong to the family of Picornaviridae (Table 1). There are three types of polioviruses. Coxsackie group A virus consists of A1 to 22, and 24, and Coxsackie group B virus consists of type 1 to 6, echoviruses group consists of types 1 to 7, 9, 11 to 27, and 29 to 34. All together, a total of 67 types are available. Starting from enterovirus type 68 to 71, they are named as enterovirus types [2]. Enterovirus type 72 is hepatitis A virus.

Historical Background

Pretissue culture period

Paralytic disease of poliomyelitis was recorded in ancient time but characterization of poliovirus was not reported until the turn of the 19th century that poliomyelitis was a viral disease. In 1909 Landsteiner and Popper transmitted paralytic disease to monkeys by inoculating them with filtered stool obtained from a patient with paralytic disease, thus demonstrating the causative agent [3]. For the next several decades, animal inoculation was the method of choice for virus isolation and study of poliomyelitis. In 1948 viruses were isolated in suckling mice that were inoculated with cell-free filtrate of stools obtained from children suffering from paralysis in the town named Coxsackie in New York [4]. These viruses were not neutralized by antisera against the three types of polioviruses. These viruses were the first group of the group A Coxsackie viruses isolated.

Post-tissue culture era

The major breakthrough for diagnosing and controlling of poliomyelitis was the discovery that poliovirus can be propagated in human embryonic tissues in cultures by Enders *et al* [5]. These cultured tissue cells allowed easy isolation of many human enteroviruses. The first of the group B Coxsackie virus was isolated in 1949 [6] from patients diagnosed as nonparalytic poliomyelitis or aseptic meningitis.

As soon as cultures of human and monkey cells began to be used for isolating polioviruses in stool specimens of patients, more unknown viruses were isolated which unlike polioviruses nor Coxsackie viruses; they were not pathogenic in laboratory animals but produced cytopathic effects in cultured cells [7]. It soon became apparent that these later agents could be isolated from healthy children as well as from patients with aseptic meningitis. Because the lack of relationship of these latter agents to human disease and because they failed to produce illness in laboratory animals, they were called "orphan" viruses or human enteric viruses, later they became known as ECHO (enteric cytopathic human orphan) viruses, a name later simplified to "echoviruses." Since certain strains of the coxsackieviruses were found to grow readily in tissue cultures and other strains failed to produce paralysis in baby mice; in addition, certain strains of echoviruses were found to be pathogenic for infant mice. As such overlapping properties accumulated, the original distinctions made between Coxsackie viruses and echoviruses were confused, it was decided that subsequent new enterovirus types would simply be assigned sequential numbers as enterovirus 68 to 71 [2]; the current accepted serotypes as listed in table 1.

Structure and Composition of Enteroviruses

Morphologically all enteroviruses are alike (Fig. 1). They are small, ether insensitive (not possessing a lipid containing envelope) viruses with a ribonucleic acid (RNA) genome. Their nucleic acid is single stranded, has a molecular weight of 2.5×10^6 d (7.7 kilo base). The nucleocapsid has a cubic (icosahedral) symmetry, and is naked. They are 20 nm to 30 nm in diameter. Virus maturation takes place in the cytoplasm.

A single molecule of the single-stranded RNA constitutes about 30% of the virion; the remainder consists of four major proteins (VP1-4) and one minor protein (VPg).

Usual Host Range of Human Enteroviruses; Animals and Cell Culture Systems

The host ranges of enteroviruses vary greatly from one type to the next and even among strains of the same type. Polioviruses have a very restricted host range among laboratory animals. Most strains will infect only Old World monkeys and chimpanzees (Table 2). Most strains can be grown in primary or continuous cell line cultures derived from a variety of human tissues or from monkey kidneys.

Coxsackieviruses are highly infectious for infant mice. All coxsackievirus group B also grow well in monkey kidney cell cultures but only certain group A viruses will grow in monkey cell cultures.

The echovirus group generally fails to produce disease in infant mice or in monkeys. However, different strains can produce variants that exhibit animal pathogenicity; for example echovirus type 9 can produce paralysis in infant mice. This variability in biological properties is the chief reason why subsequent isolates of enteroviruses are no longer being

Table 1. Classification of enterovirus

Type	Prototype strain	Illness in person yielding prototype virus	Investigator(s)
Poliovirus			
1	Brunhilde	Paralytic polio	Howe and Bodian
2	Lansing	Fatal paralytic polio	Armstrong
3	Leon	Fetal paralytic polio	Kessel
Coxsackie A virus			
1	Tompkins	Poliomyelitis	Dalldorf
2	Fleetwood	Poliomyelitis	Dalldorf
3	Olson	Meningitis	Dalldorf
4	High Point	Sewage of polio community	Melnick
5	Swartz	Poliomyelitis	Dalldorf
6	Gdula	Meningitis	Dalldorf
7	Parker	Meningitis	Dalldorf
8	Donovan	Poliomyelitis	Dalldorf
9	Bozek	Meningitis	Dalldorf
10	Kowalik	Meningitis	Dalldorf
11	Belgium-1	Epidemic Myalgia	Curnen
12	Texas-12	Files in polio community	Melnick
13	Flores	None	Sickles
14	G-14	None	Gear
15	G-9	None	Gear
16	G-10	None	Gear
17	G-12	None	Gear
18	G-13	None	Gear
19	NIH-8663	Guillain-Barre syndrome	Huebner
20	IH-35	Infectious hepatitis	Sickles
21	Kuykendall; Coe	Poliomyelitis	Lennette
22	Chulman	Vomiting and diarrhea	Sickles
24	Joseph	None	Gear
Coxsackie B virus			
1	Conn-5	Meningitis	Melnick
2	Ohio-1	Summer grippe	Melnick
3	Nancy	Minor febrile illness	Melnick
4	JVB	Chest and abdominal pain	Sickles
5	Faulkner	Mild paralytic disease with residual atrophy	Steigman
6	Schmidt	None	Hammon
Echovirus			
1	Farouk	None	Melnick
2	Cornelis	Meningitis	Melnick
3	Morrisey	Meningitis	Melnick
4	Pesascek	Meningitis	Melnick
5	Noyce	Meningitis	Melnick
6	D'Amori	Meningitis	Melnick
6'	Cox	None	Ramos-Alvarez, Sabin
6"	Burgess	Meningitis	Melnick
7	Wallace	None	Ramos-Alvarez, Sabin
8	Bryson	None	Ramos-Alvarez, Sabin
9	Hill	None	Ramos-Alvarez, Sabin
11	Gregory	None	Ramos-Alvarez, Sabin
12	Travis	None	Hammon, Ludwig
13	Del Carmen	None	Hammon, Ludwig
14	Tow	Meningitis	Melnick
15	CH 96-51	None	Ormsbee, Melnick
16	Harringiton	Meningitis	Kibrick, Enders
17	CHHE-29	None	Ramos-Alvarez, Sabin
18	Metcalf	Diarrhea	Ramos-Alvarez, Sabin

(Continued)

Table 1. *(continued)*

Type	Prototype strain	Illness in person yielding prototype virus	Investigator(s)
19	Burke	Diarrhea	Ramos-Alvarez, Sabin
20	JV-1	Fever	Rosen
21	Farina	Meningitis	Enders, Kibrick
22	Harris	Diarrhea	Sabin
23	Williamson	Diarrhea	Sabin
24	DeCamp	Diarrhea	Sabin
25	JV-4	Diarrhea	Rosen
26	Coronel	None	Hammon
27	Bacon	None	Hammon
29	JV-10	None	Rosen
30	Bastianni	Meningitis	Plager, Duncan, Lennette
31	Caldwell	Meningitis	Wenner, Lennette von Magnus
32	PR-10	Meningitis	Branche
33	Toluca-3	None	Rosen, Kern
34	DN-19	Infantile diarrhea	Melnick
Enterovirus			
68	Fermon	Lower respiratory illness	Scheibe, *et al*
69	Toluca-1	None	Rosen, *et al*
70	J670/71	Acute hemorrhagic conjunctivitis (AHC)	Kono, *et al* Yin-Murphy and Lim Mirkovic, *et al*
71	BrCr	Meningitis	Schmidt, *et al*

Modified from Melnick JL. Enteroviruses. In: Evans AS, ed. Viral Infections of Humans: Epidemiology and Control. 3rd ed. New York: Plenum Publishing Corporation,1989.

subclassified as echo or coxsackieviruses, based upon animal pathogenicity; they are named as enteroviruses with serial numbers (see discussion above). Variations in sensitivity of different cell lines to infections by enteroviruses were noted over the years by various investigators [8,9]. These variations have been used for presumptive rapid grouping of human enteroviruses in the early date [10]. Until this date many clinical laboratories still use such systems for rapid grouping of enteroviruses.

Laboratory Diagnosis

Virus isolation

Sources for isolation of enteroviruses are from feces, throat swab, cerebrospinal fluid (CSF), blood, vesicle fluid, conjunctival swab and urine. Specimens' collections are the most important initial steps for subsequent success in the isolation of viruses. The impacts of selected cell culture sensitivity need to be recognized in order to avoid obtaining false negative results.

As shown in figure 2, poliovirus induces extensive cytopathogenic effect (CPE) in both monkey kidney

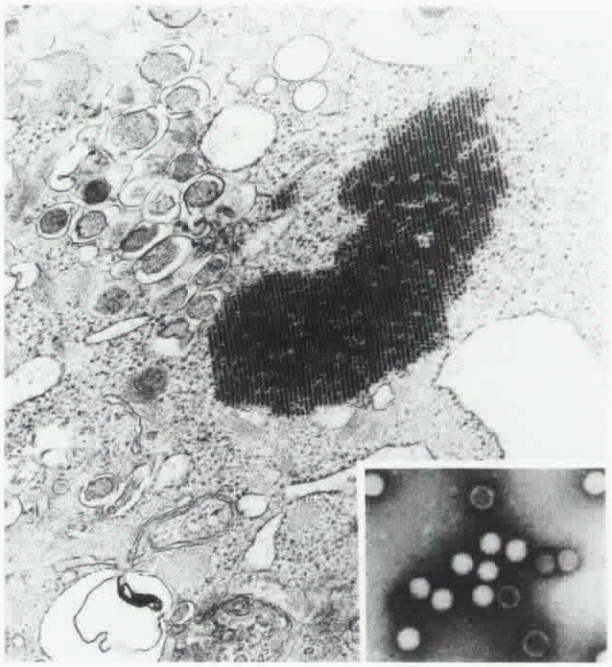

Fig. 1. Electron micrograph of poliovirus in an infected HEp-2 cell. A large aggregate of virions in crystal array in the cytoplasma (48,000 X); insert poliovirus particles stained with PTA (169,000 X) (Reproduced with permission from Hsiung, Diagnostic Virology, p. 121).

Table 2. Host range of human enteroviruses: animals and cell culture systems

Virus	Antigenic type	CPE in cell culture		Illness / pathogenicity	
		Monkey kidney cell	Human cell culture	Infant mice	Old World monkeys
Poliovirus	1-3	+	+	-	+
Coxsackie A virus	1-24[a]	±	±	+	-
Coxsackie B virus	1-6	+	+	+	-
Echovirus	1-34[b]	+	±	-	-
Enterovirus	68-71	+	+	-	-

[a]Coxsackie A 1-22,24
[b]Echovirus 1-7, 9, 11-27 and 29-34

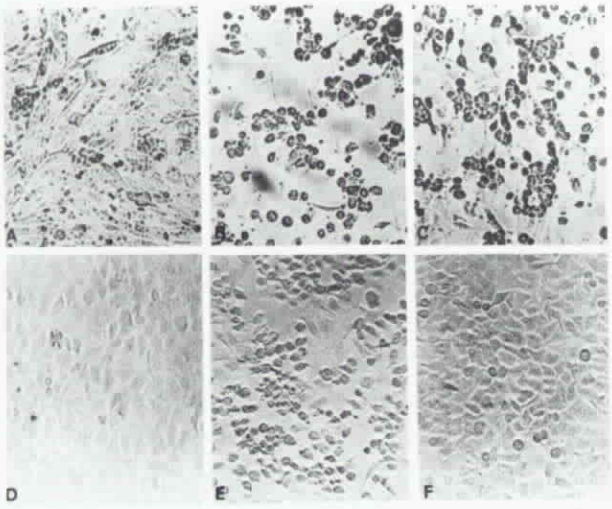

Fig. 2. Cytopathic effect induced by poliovirus 1 and echovirus 11 in RhMK and HEp-2 cultures (100 X) (Reproduced with permission from Hsiung, Diagnostic Virology, p. 124). **A.**, Uninfected RhMK culture. **B.**, CPE induced by poliovirus 1 in RhMK culture, one day after infection. **C.**, CPE induced by echovirus 11 in RhMK culture, four days after infection. **D.**, Uninfected HEP-2 culture. **E.**, CPE induced by poliovirus 1 in HEp-2 culture, one day after infection. **F.**, Absence of CPE in HEp-2 culture infected with echovirus 11, four days after infection.

cell culture and HEp-2 cell line, where as echovirus only induces CPE in monkey kidney cell culture but not in HEp-2 cell. Since the later cell culture in general is insensitive to infection with echovirus.

Laboratory methods for detection of enterovirus in central nervous system

Infections cause by enteroviruses in central nervous system (CNS) may be detected by culture, nucleic acid detection and detection of intrathecal antibody production. Early treatment is critical for minimizing neurologic damage. PCR is currently the most valuable laboratory test for noninvasive diagnosis of CNS viral infections. PCR is preferred under most circumstances, because it is positive early in the course of the disease and its sensitivity is greater than culture [11,12]. Although PCR is currently the most valuable laboratory test for noninvasive diagnosis of CNS enterovirus infections; due to the lack of standardization and limited commercial supplied reagents which are barriers to the wide spread implementation of this technique.

Final identification and serotyping of enteroviruses

Currently using monoclonal antibody by indirect immunofluorescent tests is the most efficient method for identification of some types of enteroviruses [13]. Microneutralization test in cell cultures using antiserum pool is expensive and time consuming and generally is reserved for reference laboratories. In the future, identification may be possible using molecular techniques [14,15].

Diversity in Presentations of Clinical Diseases

The incidence and prevalence of diseases associated with the enterovirus infections are based on the occurrence of a clinical syndrome sufficiently characteristic to be recognized, such as paralysis in poliomyelitis or the lesions of the hand, foot and mouth syndrome of certain enterovirus infection, or on the occurrence of an outbreak of aseptic meningitis or of an exanthema in which the causative enterovirus has been isolated. The epidemiological markers of the presence of enterovirus in the community due to enterovirus infection include the prevalence of the virus in the stools of healthy persons or in the sewage serving area, and the prevalence of antibody as determined by serological surveys. It must be kept in mind that clinical features presented by infections with different serotypes may be similar and that manifestations of infections with the same serotype may vary widely. The interdependent variables include geographic area,

climate and socioeconomic setting are important factors.

Enterovirus Circulation in Tainan, Taiwan Based on 3-year Experience (1996-1998)

The peak season for enterovirus infections varies, but generally peaks as temperatures rise. Enteroviruses are spread via the fecal-oral route with many of victims becoming ill after contact with contaminated water. Children are the most susceptible ones to enterovirus infection.

During 1996 to 1998, a total of 5872 specimens were received and examined, average 163 per month. There were 434 enteroviruses that were isolated. It is noted that most of the enteroviruses were obtained during the summer months (May to October). On the other hand, respiratory syncytial virus were recovered sporadically during the winter season (November to April) even though Tainan is located at a subtropical climate, only 22° north of the equator. The average daily temperature is 26.6 °C in the summer and 20 °C in the winter with only 6.6 °C difference between the two seasons [16]. Such observations on seasonal variations of viral activity is similar to those reported by laboratories located in temperate zone where temperatures varied greatly in the summer and the winter months.

Enterovirus 71 infection

Among the enteroviruses except poliovirus, enterovirus 71 (EV71) infections are the most important type because they are frequently complicated by the neurologic diseases including encephalitis, meningitis and epidemic poliomyelitis-like syndrome that has generated the most interest and public health implications. EV71 was first reported by Schmidt *et al* in 1974 from patients in California [17] with disease of the CNS. At which time an identical strain was recovered from the brain of a patient with fatal encephalitis; this virus was designated as EV71 [18]. In the years following 1970, EV71 outbreaks have been reported from various parts of the world including Australia [19], Sweden [20], Japan [21], Bulgaria [22], Hungary [23], Hong Kong [24], and more recently in Malaysia [25] (Table 3). It has been associated with a variety of clinical diseases including hand, foot and mouth disease, herpangina, aseptic meningitis, poliomyelitis-like paralysis and even fatal encephalitis.

Outbreak of Enterovirus 71 Infection in Taiwan 1998

In 1998 an outbreak of EV71 infections occurred in Taiwan. The outbreak had a biphasic curve with peaks in June and October, especially in the southern part of Taiwan (Liu CC, *et al*, unpublished data, 1999). There were 405 children hospitalized with hand, foot and mouth disease associated with meningitis, encephalitis or acute flaccid paralysis (AFP) and 78 have died [26]. The striking feature of this outbreak was hand, mouth and foot diseases with or without central nervous involvement (Liu CC, *et al*, unpublished data, 1999). The clinical presentation and epidemiological studies are described by Liu *et al* (Liu CC, *et al*, unpublished data, 1999). Laboratory findings indicated that the virus grew best in Vero cells and less in green monkey kidney (GMK) cells inoculated with throat swabs. No virus

Table 3. EV71 outbreak worldwide (1969-1998)

Continent	Year	Location	Clinical findings
USA	1969-1973	California	Aseptic meningitis, encephalitis
	1972	New York State	Aseptic meningitis, encephalitis Hand, foot and mouth disease
	1977	Rochester, NY	Aseptic meningitis
	1995	New Haven, CT	Neurologic, hand, foot and mouth disease
Australia	1972	Australia	Aseptic meningitis, rash
Europe	1973	Sweden	Hand, foot and mouth disease, aseptic meningitis
	1975	Bulgaria	Encephalitis, aseptic meningitis
	1978	Hungary	Aseptic meningitis, encephalitis
	1979	Lyon, France	Acute respiratory infection with CNS involvement
East Asia	1973	Japan	Hand, foot and mouth disease, aseptic meningitis
	1985	Hong Kong	Aseptic meningitis
	1987	Hubei, China	Hand, foot and mouth disease
	1997	Singapore, Malaysia	Hand, foot and mouth disease, aseptic meningitis
	1998	Taiwan	Hand, foot and mouth disease, encephalitis

was isolated from CSF (Wang JR, *et al*, unpublished data, 1999). Genetic analyses of 5'-NCR of EV71 from different clinical categories and various geographic areas showed that most of EV71 isolated during this outbreak belonged to a group of closely related clones (genotype B) and only one-tenth of isolates were in different group (genotype A) which was clustered with EV71 multiple sclerosis (MS) strain (Wang JR, *et al*, unpublished data, 1999). A comprehensive pathological and molecular study on a case of fulminant encephalitis was undertaken (Yan JJ, *et al*, unpublished data, 1999) and it was found that the sequence analysis of the new isolate and the BrCr or MS strain shared 80% nucleotide identity and 95% amino acid identity.

Control and Prevention of Enterovirus Infection

Although poliovirus infection may be eradicated from the world due to the efficient vaccination program [27], currently there is no specific antiviral agents for either treatment or prevention for other enterovirus infections. As mentioned above, poliovirus is expected to be eradicated by the year of 2000 as predicted by World Health Organization [27] but vaccines for other enterovirus are not yet available due to the many serotypes; clearly, there is a medical need for a specific antiviral agent for the management and control diseases caused by human enterovirus infections. In 1991 a new antiviral "pleconaril" which is a novel orally bioavailable and systematically acting small molecule inhibitor for picornaviruses (including enterovirus and rhinovirus) [28]. The latter is currently in clinical trials for treatment of enterovirus meningitis and respiratory infections.

Acknowledgments

The author would like to thank National Health Research Institutes for the grants of NHRI-CN-CR8801S and NHRI-CN-CR8804P (National Health Research Institutes Contract Research).

References

1. Melnick JL, Agol VI, Bachrach HL, Brown F, Cooper PD, Fiers W, Gard S, Gear JH, Ghendon Y, Kasza L, LaPlaca M, Mandel B, McGregor S, Mohanty SB, Plummer G, Rueckert RR, Schaffer FL, Tagaya I, Tyrrell DA, Voroshilova M, Wenner HA. Picornaviridae. Intervirology 1974;4:303-16.
2. Melnick JL, Tagaya I, von Magnus H. Enterovirus 69, 70 and 71. Intervirology 1974;4:369-70.
3. Landsteiner K, Poppe E. Landsteiner and discovery of the virus. In: Paul JR, ed. A History of Poliomyelitis Virus. New Haven: Yale University Press, 1971:98.
4. Dalldorf G, Sickles GM. An unidentified filterable agent isolated from the feces of children with paralytic poliomyelitis or aseptic meningitis. Science 1948;71:108:61-3.
5. Enders JF, Weller TH, Robbins FC. Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissue. Science 1949;109:85-7.
6. Melnick JL, Shaw EW, Curren EC. A virus isolated from patients diagnosed as non-paralytic poliomyelitis or aseptic meningitis. Proc Soc Exp Biol Med 1949;71:344-9.
7. Robbins FC, Enders JF, Weller TH, Florentino GL. Studies on the cultivation of poliomyelitis viruses in tissue culture. V. The direct isolation and serologic identification of virus strains in tissue from patients with non-paralytic and paralytic poliomyelitis. Am J Hyg 1951;54:286-93.
8. Dagan R, Menegus MA. A combination of four cell types for rapid detection of enteroviruses in clinical specimens. J Med Virol 1986;19:219-28.
9. Kok TW, Pryor T, Payne L. Comparison of rhabdomyosarcoma, buffalo green monkey kidney epithelial, A 549 (human lung epithelial) cells and human embryonic lung fibroblasts for isolation of enteroviruses from clinical samples. J Clin Virol 1998;11:61-5.
10. Hsiung GD, Melnick JL. Differentiation of poliomyelitis, Coxsackie and ECHO (Enteric cytopathogenic human orphan) viruses by plaque morphology and host cell susceptibility. Fed Proc 1956;15:1932.
11. Rotbart HA. Diagnosis of enteroviral meningitis with the polymerase chain reaction. J Pediatri 1990;117:85-9.
12. Chapman NM, Tracy S, Gauntt CJ, Fortmueller U. Molecular detection and identification of enteroviruses using enzymatic amplification and nucleic acid hybridization. J Clin Microbiol 1990;28:843-50.
13. Rigonan AS, Mann L, Chonmaitree T. Use of monoclonal antibodies to identify serotypes of enterovirus isolates. J Clin Microbiol 1998;36:1877-81.
14. Muir P, Kammerer T, Korn K, Mulders MN, Poyry T. Molecular typing of enteroviruses; current status and future requirements. Clin Microbiol Rev 1998;11:202-27.
15. Oberste MS, Maher K, Kilpatrick DR, Flemister MR, Brown BA, Pallansch MA. Typing of human enteroviruses by partial sequencing of VP1. J Clin Microbiol 1999;37:1288-93.
16. Wang JR, Kuo PH, Yee JJ, Chen MY, Su IJ. Seasonal variations of viral activity in Tainan, Taiwan based on two years experience [abstract]. Proceedings of the 13th Annual Clinical Virology Symposium, April 27-10, 1997.
17. Schmidt NJ, Lennette EH, Ho HH. An apparently new enterovirus isolated from patients with disease of the central nervous system. J Infect Dis 1974;129:304-9.
18. Melnick JL. Enterovirus type 71 infections: a varied clinical pattern sometimes mimicking paralytic poliomyelitis. Rev Infect Dis 1984;6:387-90.
19. Kennett ML, Birch CJ, Lewis FA, Yung AP, Locarmini SA, Gust ID. Enterovirus type 71 infection in Melbourne. Bull WHO 1974;51:609-15.
20. Blomberg J, Lycke E, Ahlfors K, Johnsson T, Wolontis S, von Zeipel G . New enterovirus type associated with epidemic of aseptic meningitis and /or hand, foot and mouth disease. Lancet 1974;2:112.
21. Tagaya I, Tachibana K. Epidemic of hand, foot and mouth disease in Japan 1972 and 1973: difference in epidemiologic and virologic features from previous one. Japan J Med Sci Biol 1975;28:231-4.
22. Chumakov M, Voroshilova M, Shindarov L. Enterovirus 71 isolated from cases of epidemic poliomyelitis-like disease in

Bulgaria. Arch Virol 1979;60:329-40.
23. Hagy G, Takatsy S, Kukan E, Mahay I, Domok I. Virological diagnosis of enterovirus type 71 infections: experiences gained during an epidemic of acute CNS disease of Hungary in 1978. Arch Virol 1982;71:217-27.
24. Samuda GM, Chang WK, Yeung CY, Tang PS. Monoplegia caused by enterovirus 71: an outbreak in Hong Kong. Pediatr Infect Dis 1987;6:206-8.
25. Lum LCS, Wong KT, Lam SK. Fatal enterovirus 71 encephalomyelitis. J Pediatri 1998;133:795-8.
26. Ho M, Chen ER, Hsu KH, Twu SJ, Chen KT, Tsai SF, Wang JR, Shih SR. An epidemic of enterovirus 71 infection in Taiwan. N Engl J Med 1999;341:929-35.
27. World Health Assembly global eradication of poliomyelitis by the year 2000. Geneva: WHO 1988 resolution WHO 11-28.
28. Pevera DC. Antiviral therapy for picornavirus infections: Pleconaril [abstract]. Proceedings of the 15th Annual Clinical Virlogy Symposium, May 9-12, 1999.